THE APPLICABILITY OF BATCH TESTS TO ASSESS BIOMETHANATION POTENTIAL OF ORGANIC WASTE AND ASSESS SCALE UP TO CONTINUOUS REACTOR SYSTEMS

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CONTENTS

ABSTRACT

Many of the current methods of assessing anaerobic biodegradability of solid samples require sample modification prior to testing. Steps like sample drying, grinding, re-drying and re-grinding to 2mm or less make the test results difficult to apply to field conditions and could lead to oxygen exposure, possibly distorting the results. Finally, because of a small sample size of about 10-50g w/w, the test result may not be representative of the bulk material.

A new tool dubbed 'tube' has been developed, made of 10 cm ∅ PVC pipe measuring 43.5 cm long with 3600 ml capacity with caps at both ends. For easy sample introduction, one endcap is fixed while the other is screw capped. A distinctive feature is the wide neck opening of about 10 cm where solid samples can be introduced as is, without further sample modification.

Research has proven the tube applicable across various types of solid organic waste and conditions provided that a suitable organic loading rate is determined. The tube is best operated using 5-7 days pre-digested digested sewage sludge as seed, with minimal mixing and without the addition of nutrients or alkali solution. The test result can be obtained within 4-6 days to 20 days, signifying a 50-75% and 95% substrate degradation, respectively. Irreproducibility seen in some experiments may not only be a function of the seed and the substrate. The organic loading rate (OLR) at which the test is conducted is also influential especially if test is conducted closer to its maximum OLR tolerance where anaerobic process is more erratic.

The performance of a continuous reactor digesting on a similar substrate can be estimated using this new tool. Food waste is established by the tubes to have an ultimate methane potential (B0) of 0.45L CH_4/g VS. The same substrate when digested in a continuous reactor will produce about (B) 0.32 L CH_4/g VS. The first order rate constant for both systems; batch

and continuous are identical at 0.12 to 0.28 d^{-1}. First order kinetics is efficient at modelling the anaerobic degradation when the process is healthy but may be less reliable under an unstable process.

This research recommends the use of kinetics in combination with the experimental data (e.g. HRT, OLR, yield) when planning and designing an industrial plant to avoid overdesign and unnecessary building, maintenance and operating costs.

DEDICATIONS

Thank you God, for the opportunities that have brought me where I am today. For giving the strength to keep on striving when I am in despair. And for meeting me with such tremendous and inviduals who helped and supported me throughout my time here in New Zealand.

This thesis is especially dedicated to my loving husband, Rojali. Thank you ayah, for the love and care you have poured me, Haikal and Arissa. For the support and endurance you have shown, making this thesis much easier to be completed. Our journey here has only strengthened my love for all of you.

I am immensely appreciative of the support and encouragement by my parents, family and in laws in Malaysia, in meeting this challenge. Thank you and may God repay your kindness. Also, to all my friends, we have shared good times and difficult memories, which will remain with me forever.

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CHAPTER 1

INTRODUCTION

1.1 SUSTAINABLE MANAGEMENT OF WASTE THROUGH ANAEROBIC DIGESTION

The challenge for the 21st century in terms of a sound waste management strategy is the transformation of waste into resources for the future (Lens et al., 2004). Utilization of proper options for the treatment of organic wastes can turn this burden into an asset. One way of achieving this is by employing biological waste treatment methods. Anaerobic digestion is an example of an emerging method, offering avenues to recover both energy through anaerobic process and compost whilst reducing waste at the same time.

Anaerobic digestion is the biological decomposition of organic substrates in the absence of oxygen. In anaerobic treatment, its benefits, advantages, limitations and requirements, which are unique to each substrate, are often dictated by its biodegradability. Understanding substrate biodegradability hence becomes important when designing or operating a waste treatment plant. Certain operating parameters such as temperature or mixing and substrate related factors like loading rate and pretreatments may be better suited than others and this might form a logical limit on the usefulness of a given approach. In response to this question, research at laboratory level is usually conducted beforehand to pinpoint suitable operating conditions and foresee any technical or toxicity issues with a waste sample.

1.2 AN OVERVIEW OF THE ANAEROBIC DIGESTION PROCESS

When an organic material enters into a digester, it contains a large fraction of suspended solids and complex soluble matter. Under anaerobic conditions, these large organic molecules are converted, mainly, into methane (CH_4) and carbon dioxide (CO_2) by the action of bacteria. The overall biochemical reaction can generally be simplified to:

$$\text{Organic material} \longrightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S$$

(Evans, 2001)

The process of anaerobic digestion proceeds in three main stages; (i) hydrolysis, (ii) acid formation and (iii) methanogenesis (refer Figure 1.1).

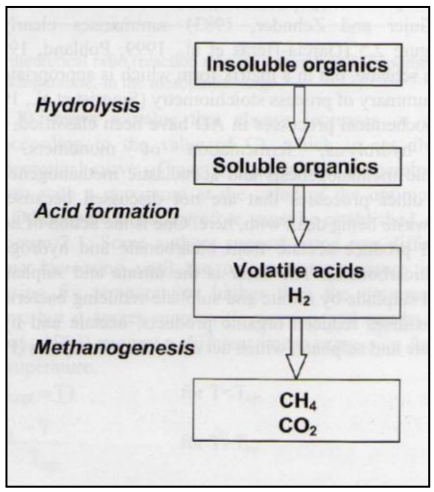


Figure 1.1 Anaerobic digestion described by three processes

(source: Mata-Alvarez, 2003)

1.2.1 Hydrolysis

Hydrolysis is the first step in the anaerobic biodegradation process. It involves the conversion of the complex waste (particulate and soluble polymers) into soluble products by the extracellular enzymes secreted by the hydrolytic bacteria. The once complex insoluble organic polymers become more easily available for use by the acidogenic bacteria in the next stage. Proteins present in the waste are converted into amino acids, fats into long chain fatty acids and carbohydrates into simple sugars.

Hydrolysis has been reported to usually be the rate-limiting step, since bacterial action at this stage proceeds slower than the other following digestion steps. Substrate availability, bacterial population density, temperature and pH (Chynoweth and Isaacson, 1987; Palmisano and Barlaz, 1996; Evans, 2001) all affect the rate at which hydrolysis proceeds. The competence of the hydrolysis stage plays a huge role in determining the substrate ultimate methane yield. A municipal solid waste (MSW) for example only underwent 50% hydrolyzation of its organic matter, while the rest remained undegraded. This phenomenon occurred because certain sites within the solid matrix are inaccessible to the hydrolysis enzymes and due to shortage of appropriate organisms secreting the necessary extracellular enzymes (Palmisano and Barlaz, 1996).

1.2.2 Acid formation

1.2.2.1 Acidogenesis

In acidogenesis, the organic monomers of sugars and amino acids released earlier are degraded by the fermentative bacteria to produce volatile fatty acids (VFA) namely propionic, butyric and valeric acids, together with acetate, hydrogen (H_2) carbon dioxide (CO_2). The degradation of amino acids also produces ammonia (NH_3).

1.2.2.2 Acetogenesis

During the acetogenesis step, the obligate hydrogen producing acetogens (OHPA) break down both long chain fatty acids (LCFA) and volatile fatty acids (VFAs), producing acetate, carbon dioxide and hydrogen. LCFA are organic acids having more than 5 atoms of carbon (Angelidaki et al., 1998). Carbon dioxide is produced when fatty acids as electron donors degrades, at the same time, as electron acceptors, transforming H^+ into H_2 (Mata-Alvarez, 2003).

1.2.3 Methanogenesis

Methane is produced from the raw materials of the previous stage during the methanogenesis stage. This is done via two ways; one through hydrogenotrophic methanogenesis producing methane by utilizing H_2 and CO_2 by the hydrogen-consuming bacteria in a syntrophic co-culture with the OHPA bacteria. The other by methanogenic aceticlastic bacteria which grow on acetate as substrate, releasing methane and carbon dioxide. Of these, acetic acid (CH_3COOH) and the closely related acetate are the main precursors to methane production accounting to about 75% of the methane production, as depicted in the following equation:

$$CH_3COOH \longrightarrow CH_4+CO_2$$

Methane forming bacteria may also use methanol:

$$CH_3OH+H_2 \longrightarrow CH_4+H_2O$$

or carbon dioxide and hydrogen:

$$CO_2+4H_2 \longrightarrow CH_4+2H_2O$$

Source: Evans, 2001

The evolution of the biogas coincides with the cellulose decomposition and is better illustrated with curves going through five main phases, as described below (source: Evans, 2001):

Phase I

Maximum cellulose loadings; oxygen content drops to near zero; carbon dioxide at atmospheric levels (78%)

Phase II

Carbon dioxide, hydrogen and free fatty acids levels rise to peak values; cellulose breakdown begins

Phase III

Carbon dioxide decreases to plateau at around 40%; methane production commences and achieves plateau at around 60%; free fatty acids decrease to hold at minimal levels; cellulose breakdown continues at a linear rate with respect to time

Phase IV

Plateau phase with carbon dioxide at circa 40% methane at circa 60% and free fatty acids at less than 5%; cellulose declines steadily throughout this phase

Phase V

Cellulose becomes fully decomposed, resulting in the tail off to zero of methane and carbon dioxide; oxygen regain atmospheric levels (20%).

1.2.4 Process balance

When the rate of hydrolytic and fermentative activity exceeds the rate of acetogenic conversion of fermentation intermediates, process inhibition occurs since methanogens cannot consume dihydrogen at the rate at which it is produced. When methanogenesis is inhibited, electron or hydrogen accumulates. This results in the formation of various other fermentation products like propionate, butyrate, lactate, succinate, and alcohols, wanting to rid of the electrons or hydrogen. The reactor pH then decreases as the levels of this fermentation products continue rising. The methane forming bacteria are pH sensitive and can only usually tolerate more or less neutral pH values of between 6.5 to 7.5.

If the fermentation products accumulate to inhibitory levels, the decomposition of substrate will cease. It is therefore very important to understand that the overall fermentation is dependent largely on the particular bacteria species present and to a lesser extent its environmental conditions. There has to be a balance between activities of bacteria that form organic acids, carbon dioxide, and dihydrogen (or formate) and the methanogenic bacteria which utilize these substrates. By changing VFAs into methane and other related gasses, any inclination towards an augmentation in VFA concentration which simultaneously decreased the digester pH, is removed. The acid/base equilibrium is hereby naturally regulated, removing the likelihood for biochemical inhibition and/or bacterial population destruction due to an acidified digester environment.

1.2.5 Kinetics

The three major anaerobic degradation processes described earlier, namely the hydrolysis, acidogenesis/acetogenesis and methanogenesis can be modelled, providing theoretical understanding of the microbial populations and their interactions in the environment within the reactor. Not only can the reactor design and operation be optimised, the kinetic equation is particularly useful as guidelines for scale-up of the digestion systems.

For different substrates, the kinetics are different; and for each substrate the kinetics vary with operating conditions. Numerous kinetic models have been developed to describe the degradation steps involved such as Monod, Step Diffusion, Inhibition and Shrinking Core (Chynoweth and Isaacson, 1987; Mata-Alvarez, 2003). The simplest rate expression is the first-order kinetic model since only a single parameter needs to be estimated. Although sometimes limited, especially where complex substrate and systems are involved, the first-order model is generally capable at predicting the anaerobic process with a great degree of reliability. Considering the importance of process kinetics to this research as well as the vast information the topic itself carries, it seems more appropriate to conduct a thorough review. Further description and discussion of the biodegradation yields and kinetics as an essential tool for rational reactor design is allocated in Chapter 2: Literature review.

1.3 MEASURING ANAEROBIC DECOMPOSITION

Anaerobic biodegradability can be ascertained by the amount of substrate transformed or the intermediates and end products developed by the different microorganisms groups. The volume of biogas produced also gives an indication of anaerobic decomposition. A number of methods are available to measure anaerobic biodegradability. Although these techniques vary in terms of the test procedure, measuring system and results, they can generally be simplified into three categories. This classification is based on the equipment used to measure anaerobic biodegradability and activity including those that use (i) a variety of chemical analysis techniques to quantify substrate depletion or the formation of intermediates and end products, and (ii) microbiological based methods and (iii) gasometric measurement techniques to measure the amount of biogas produced.

1.3.1 Analysis of substrate consumption or product formation

1.3.1.1 COD, TOC, DOC

Substrate concentrations can be quantified by using either a summary or a substrate-specific parameter. The dissolved organic carbon (DOC), chemical oxygen demand (COD) and total organic carbon (TOC) are generally applicable to assess the consumption of the whole substrate. However, the evolution from one component to another by anaerobic biodegradation may affect only a small change in COD, TOC or DOC, making the change harder to detect. Moreover, the complex nature of the test sludge and the tendency of some test chemicals to absorb to sludge particulate material may upset the suitability of the method to monitor substrate conversion (Birch et. al., 1989). That said, this does not mean that these gross measurements (as well as volatile solids, VS) are incapable of telling when there is substrate conversion to gas (CO_2/CH_4).

1.3.1.2 Uniformly ^{14}C labeled test substances

In the investigation of benzene degradation by Burland and Edwards (1999), radioactively labeled ^{14}C was used and monitored by scintillation counting of radioactivity. The ^{13}C-labelled and ^{14}C-labelled substances have also been followed using gas chromatography-mass spectrometry (GC-MS) as a way of quantifying the mass balance between substrate and products (Dolfing and Bloemen, 1985). Tests using these radioactively labeled substances involve the detection of radioactive methane and carbon dioxide to present clear indication of ultimate biodegradation. While this test is well validated, the labeling approaches of its test procedure limits the test's widespread applicability, especially when screening new chemicals. Labelling approaches involve adding a ^{13}C-labelled substrate to the substrate or alternatively by using highly ^{13}C-labelled substrates produced by raising plants or algae on $^{13}CO_2$ or $H^{13}CO_3^-$, respectively.

Another approach is to employ ^{13}C-labelled substrates produced by organic synthesis. A wide range of ^{13}C-labelled compounds are available from commercial suppliers. The ^{13}C-labelled synthetic compounds can be expensive, thereby restricting their use in field scale studies (Evershed et al, 2006).

1.3.1.3 Coenzyme F_{420}

Methanogens induce UV blue-green autofluorescence, quantifiable under an autofluorescence microscopy. Methanogens with a high content of F_{420} such as the hydrogen-utilising methanogens are visible. Exceptions apply to some methanogens which are not visually clear such as the acetate-utilising methanogens that cannot be counted at all or the genus *Methanosarcina* which are found in clumps made of many individual cells (Solera et al., 2001a).

A high correlation was evident between the methane production and methanogenic population in thermophilic anaerobic reactors using autofluorescence microscopy as the method of analysis (Solera et al., 2001b). This method benefits over traditional microbiological methods as it can provide predictive value of the sample's potential methanogenic activity without the need for culturing. A later study by Demirel and Yenigun (2006) found that numbers of autofluorescent methanogens decreased during start-up. The type of substrate used and/or the variations of the organic loading rate (OLR) may have affected the morphology of the autofluorescent methanogens during start-up.

The technique is not capable for distinguishing between live and dead microorganisms. In addition, the accuracy of results is easily influenced by fading of the autofluorescent cells and the presence of weakly or non-fluorescent cells (Solera et al., 2001a).

1.3.2 Analysis based on microbiological methods

The molecular biology approach is based on the RNA of the small ribosomal subunit (S rRNA for prokaryotes) or their corresponding genes. The RNA molecule was chosen due to its obvious advantages, namely (i) being universal and abundant in all living beings (10^3 to 10^5 ribosomes/cell) (ii) molecule highly preserved throughout evolution (iii) sufficiently long gene sequence to produce statistically relevant data and (iv) gene can be easily sequenced with current technology (Sanz and Kochling, 2007). The current techniques most widely used include cloning of 16S rDNA, denaturant gradient gel electrophoresis (DGGE) and Fluorescent in situ hybridization (FISH).

1.3.2.1 Cloning of 16S rDNA

Cloning and sequencing for 16S rDNA is the most widely used procedure in molecular biology. The technique involves nucleic acids extraction, then magnification and cloning of the 16S rDNA genes. The genes are later sequenced and the isolated clone identified using phylogenetic software (Figure 1.2).

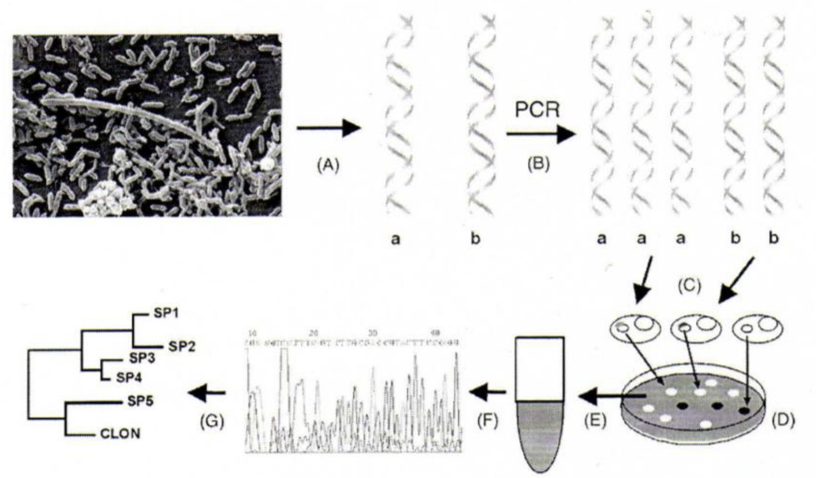


Fig. 1. Outline of the cloning procedure for studying a microbial community. The work cycle is as follows: (A) direct nucleic acid extraction, without the need for previous isolation of microorganisms; (B) amplification of the genes that code for 16S rRNA by polymerase chain reaction (PCR), commonly using universal primers for bacteria or archaea, resulting in a mixture of rDNA copies corresponding to the microorganisms present in the sample; (C) cloning of the PCR products obtained into a suitable high copy number plasmid and transformation of competent *E. coli* cells with this vector; (D) selection of transformed clones with an indicator contained in the plasmid (the white colonies in the figure); (E) extraction of plasmid DNA; (F) sequencing of the cloned gene, creating a clone library; (G) determination of the phylogenetic affiliation of the cloned sequence with the help of dedicated computer programs (ARB, Seqlab, PAUP, PHYLIP).

Figure 1.2. Outline of the cloning procedure for studying a microbial community (source: Sanz and Kochling, 2007)

Mladenovska et al., (2003) was able to identify the microbial communities affecting the performance of two anaerobic reactors treating cattle manure and a mixture of cattle manure and lipids, respectively through cloning of 16S rDNA. The technique revealed that archaeal species were restricted in both reactors with most clones related to the *Methanosarcina sicilae.* Presence of sequences related to the species *Methanosarcina sicilae* in both reactors indicated that the same acetate-utilizing methanogens were active in conversion of higher amounts of acetate generated during the degradation of LCFA. The cloning approach was also applied to characterize the microbial properties of a methanogenic granular phenol-degrading sludge in the work by Zhang et al. (2005). Together with FISH, a detailed picture of the microbial population especially the species responsible for phenol degradation was better identified.

Cloning of 16S rDNA yields far more exact phylogenetic information than other molecular techniques such as FISH and DGGE, however, it is less accepted in the fields of environmental engineering. Demands for specialized personnel and equipment not necessarily available in engineering and chemistry departments may be the reason behind this lack of popularity. The cloning method also can be time consuming and cumbersome, making it unpractical for high sample thoroughput (Sanz and Kochling, 2007).

1.3.2.2 Denaturant gradient gel electrophoresis (DGGE)

Denaturant gradient gel electrophoresis (DGGE) helps determine the dominant members of a microbial community with the medium phylogenetic resolution without having to go through a laborious method as the cloning of 16S rDNA. This is generally so if knowledge of the exact phylogenetic information is less desirable.

With the DGGE technique, band patterns that directly reflect the genetic biodiversity of the sample are generated, following the various mobility on a gel of denatured DNA-fragments of similar size but with different nucleic acid sequences. The procedure is described in Figure 1.3.

When DGGE analysis was used for studying the effect of various dilution rates on carbohydrate fermentation in a continuous flow stirred tank reactor, a population shift in the microbial community occuring with changing dilution rate (Ueno et al., 2001) was exposed. Majority of the microorganisms population detected were that of closely related thermophilic anaerobic bacteria. Another study demonstrated the applicability of DGGE for showing impact of temperature on bacterial community structure and diversity for bioreactors treating pharmaceutical wastewaters (Lapara et al., 2000). Three distinct microbial communities were observed with a reduction in number of bacterial populations seen under elevated, thermophilic reactor temperature.

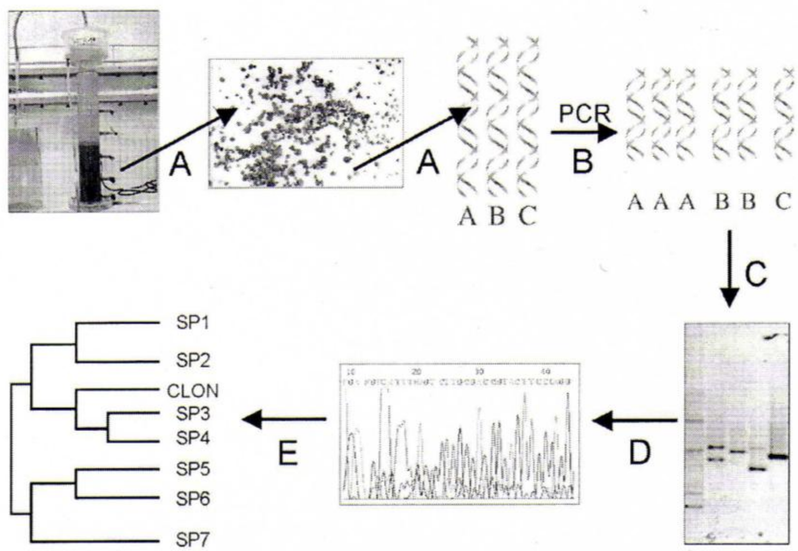


Fig. 2. Schematic representation of DGGE. DNA is extracted from the original sample, in this case as granular sludge from a UASB reactor (A). The 16S rRNA gene is partially amplified by PCR usually with universal primers to give a mixture of DNA fragments, all of the same length. Each of the different product DNA molecules resulting from this step represents a different microorganism (B). The DNA mixture is then separated by denaturant gradient electrophoresis on an acrylamide gel with an increasing urea/formamide gradient. The DNA molecules migrate towards the positive pole and come to a halt on the gel on reaching their corresponding denaturant force (Tm), which depends on the DNA sequence. Every band on the gel corresponds to a different microorganism in the sample, providing sufficient information for many requirements (C). The bands can be cut from the gel and the DNA extracted and sequenced (D). Comparison of the sequences with a 16S rDNA database allows to determine the phylogenetic affiliation of the microorganism (E).

Figure 1.3 Schematic representation of DGGE (source: Sanz and Kochling, 2007)

The DGGE technique is very valuable for following dynamic modification in microbial communities, especially involving a large number of samples. It allows a rapid and simple examination of band patterns. However, the small number of detected bands restricts the number of identifiable species. The phylogenetic relations are thus less reliable, since the sequences of the bands involve only a small region (200-600 bp) of the 16S rRNA gene.

1.3.2.3 Fluorescence in situ hybridisation (FISH)

Fluorescence in situ *hybridisation* (FISH) is a technique that uses fluorescently labeled phylogenetic oligonucleotide hybridisation probes to detect specific microbial groups. The microbial cells having 16S rRNA sequences are hybridized with the fluorescent-dye labeled probes and detected under a microscope. The microbial abundance and/or activity are determined from the extent of hybridization (Figure 1.4).

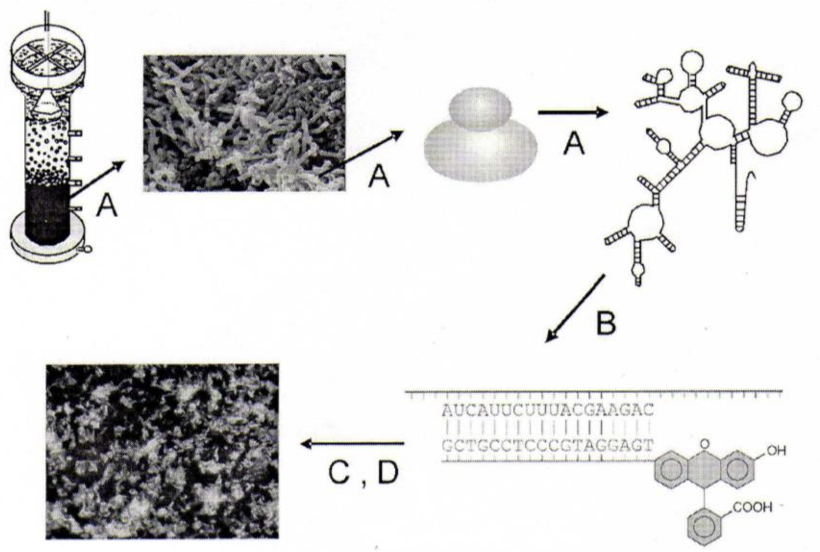


Fig. 3. The *in situ* hybridization process can be divided in four stages: (A) sampling and immediate fixation in formaldehyde to preserve the integrity of the cells, especially the ribosomes; (B) hybridization with a specific probe, labelled with a fluorescent dye at its 5′-end and matched with a sequence of the 16S rRNA; (C) counterstaining with a universal marker (DAPI, which attaches non-specifically to DNA molecules) or another more general probe, labelled with a different fluorescent dye; (D) visualization via fluorescence microscopy. Direct quantification is possible by manual counting of hybridized cells (epifluorescence and laser confocal microscope) or by image analysis of digital photos (both microscopes) or automated counting with a flow cytometer. The ratio of cells that have hybridized with the specific probe to the total cell count with DAPI or a more general probe gives the percentage of bacteria present in the sample, based on the total number of cells or on the group that was chosen with the general probe (e.g. bacteria or archaea).

Figure 1.4 The in situ hybridization process described in four stages
(Sanz and Kochling, 2007)

Stahl et al. (1995) have earlier distinguished probes suitable for identifying methanogenic microorganisms at different taxonomic levels (order, family and genus). Their work sets the ground for further application of FISH in the area of anaerobic digestion. Such applications include the determination of methanogenic activity in thermophilic-dry anaerobic reactors by comparing the amount of methane generated with the size of methanogenic population (Montero et al., 2008). The capability of FISH to illustrate the effects of high free ammonia concentrations on the performances of anaerobic bioreactors has also been verified (Calli et al., 2005). The propionate degrading acetogenic bacteria were more sensitive to free ammonia than methanogenic archea.

The test itself is suitable for routine analysis as it is fast and easy to perform without use of highly trained and specialized personnel. However, problems may arise if the probe for the desired bacterial taxon or group is not available. In addition, the user needs to be familiar with the target microorganisms, its ecosystem and the rRNA sequence is necessary prior to using FISH technique.

1.3.3 Gasometric measurement techniques

The gasometric methods, or sometimes termed respirometer, have been repeatedly applied for determining anaerobic biodegradation (Birch et al., 1989; Colleran et al., 1992; Guwy, 2004). Gasometric methods incorporate a simple apparatus: (i) the reactor and its associated contents and (ii) gas quantification system to determine the amount of biogas produced as a result of microbial activity in a given sample.

1.3.3.1 The reactor

The most common reactor configurations for anaerobic degradability testing in the laboratory are the batch and continuous type. The reactors differ in terms of their feeding modes, effluent analysis, test period as well as the kinetics, and are generally chosen depending on the objective of the study.

In the batch approach, the substrate is incubated with inoculum and may be supplemented with a buffer solution and nutrients. The batch operation is characterized by no addition to and withdrawal from the reactor after initiation until the end of the incubation period. If the reactor effluent needs to be analysed over time, a system where several reactors is used is usually the most suitable option. Several identical reactors are set-up at the start and assumed to represent one large reactor undergoing similar biodegradation process over time. For effluent analysis, one whole reactor is sacrificed.

The continuous set-up uses stirred tank reactors with analysis made on the effluent once steady state is established. Here, nutrients essential for growth are continuously fed. A portion of the reactor contents is continuously withdrawn and exchanged with equivalent amount of fresh substrate.

The continuous procedures closely simulate full-scale anaerobic operation; however, they are costly in terms of facilities, equipment, time and personnel. Long term operation of continuous processes are prone to many operating difficulties, such as contamination and cell washout causing decrease in activity. It is also susceptible to unanticipated disturbances like temperature failure and feedstock variability. The batch operation is not restricted by the same weaknesses as the continuous reactors. Batch procedure allows the evaluation of a wide range of variables; however, there is still room for further improvement. The advantages, flexibility, capabilities and kinetics of each reactor configuration in realising anaerobic biodegradation potential of solid organic waste is examined even more in the succeeding chapter (Chapter 2: Literature review).

1.3.3.2 Gas quantification system

The volume of biogas generated is determined either through *volumetric* or *manometric* means. The former measures the biogas volume by keeping the pressure constant while the latter measures the pressure increase by keeping the volume constant.

a) Volumetric methods

In volumetric methods, the biogas produced is allowed to move into an external collection system that measures the volume produced. This may be as simple as a lubricated glass syringe that is inverted straight onto the lid of the reactor, first introduced by Owen et al. in 1979. The overpressure generated inside the reactor forces the piston to expand, balancing the pressure buildup to atmospheric pressure. The volume of biogas is read off straight and then either injected back into the serum bottle, or discarded to the atmosphere.

Alternatively, liquid displacement device can be employed. The set-up usually consists of a suitable vessel filled with a barrier solution and inverted in a reservoir. The biogas from the reactor passes to this liquid filled vessel thus displacing an equivalent liquid volume. The amount of biogas is determined either by graduation or by weighing the weight of liquid displaced.

The Eudiometer unit is a standardized example of a manual volumetric method based on the liquid displacement concept (see Figure 1.5). Reading is taken off the graduated gas collecting tube, after the barrier solution is displaced into the reservoir tank. To bring the displaced liquid to its original level (liquid levels in both the gas collecting tube and the reservoir tank are similar), the reservoir tank is raised so that the biogas returns to atmospheric pressure.

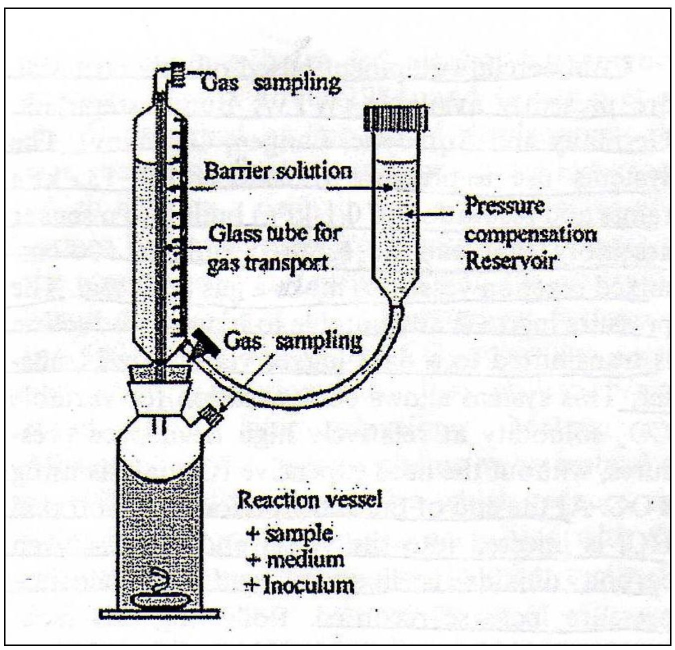


Figure 1.5 Eudiometer unit (adapted from Guwy, 2004)

Most of the above mentioned techniques can be run automatically. The automation usually relies on the container reaching a pre-set level which will then activate a sensor. This sensor then triggers a solenoid valve (two way or three way), releasing the accumulated gas to the atmosphere and resets the whole system. At the same time, the counter rotates or ticks to account for one unit volume of gas produced. The cycle will repeat itself thus begins another count. Recent example of this automated liquid displacement concept utilizes a volumetric cell for gas-liquid displacement and optical couplers as sensors for liquid level detection (Liu et al, 2004). An alternative design prefers gas accumulation which is regulated by a counter-weighted floating cap and the level controlled by a pressure sensor (Smith and Stockle, 2008). Kuss and Young (1992) invented a bubble counter, where gas bubbles of very small volumes of gas passed through a photocell and a detector count the number of bubbles formed. These new inventions are a result of ongoing modifications from previous developed devices such as the ones used by Cooney (2006), Glauser et al. (1984), Gwatkin et al. (1986), Liu et al. (2004) and Veiga et al. (1990). The vast number of gas measurement designs points out that no single device is suitable for all researchers governed by the availability of local supplies, funding and the research requirements.

b) Manometric Methods

Under manometric means, the pressure build up is measured from the biogas being confined in the reactor itself. Pressure increase can be traditionally measured using a differential manometer, however the work by Shelton and Tiedje (1984) sets the ground for applicability of pressure transducers in quantifying biogas production. Pressure transducers have been widely accepted ever since as they can be installed within the reactor, allow easy automation and amenability to computer control.

Various configurations of the pressure transducer techniques are available. Generally, the set-up depends on the pressure inside the reactor reaching a set value and sending an electric signal to a control interface that activates a three-way solenoid valve. The second valve will shut to maintain the internal pressure of the reactor while the third valve will open to release excess pressure to the atmosphere. After a set time period (about 3 seconds), the valve will deactivate and a new cycle begins. The system employed by Ince et al. (1995) incorporates the use of a microcomputer which enabled monitoring of 8 independent reactors simultaneously. Coates et al. (1996) used a portable pressure transducer device for determining hydrogenotrophic activity of anaerobic sludges and found the method to be comparable with the conventional gas chromatographic measurement of the biogas composition. Angelidaki et al. (1998) also described a computerized system simultaneously monitoring 16 reactors with the results validated against a gas chromatographic analysis.

The water displacement devices which make up the majority of volumetric gas metering methods are cheap and involve simple laboratory apparatus like water cylinders and plastic tubings. The manual approach is often cumbersome and time consuming making extended incubation period harder. This is due to the need for constant evacuation and resetting of the water level as large gas volumes cannot be catered by the small reservoir liquid available for the volume displacement. The automatic approach is less labour intensive but is more prone to problems associated with reservoir levels, corrosion, and complexity in managing CO_2 solubility and algae growth (Guwy, 2004). Corrosion is especially an issue with electronic solenoid valves and may be lessened using a siphon valve instead. The bubble counter is easily influenced by the liquid volume and viscosity where any variation may affect the bubble size thus the volume measured.

The pressure transducer can yield very accurate output and is easier to perform than the liquid displacement techniques, although more effort is required in its initial set-up. However, the technique is more susceptible to pressure rise with the temperature and gas-to-liquid volume ratio highly influencing the measurement. Temperature deviations may cause background fluctuations, decreasing the sensitivity of a pressure transducer. On the other hand, the sensitivity of the technique is enhanced when a low gas-to-liquid volume meaning a reduced headspace volume is opted.

Regardless of which gasometric method, whether volumetric or manometric, the dissolution of CO_2 into water and its effects to the measurement is a recurring phenomenon. It is widely accepted that a considerable fraction of carbon dioxide can dissolve in water while methane hardly does. Factors such as pressure, pH, ratio of headspace-to-liquid volume, temperature and the complex thermodynamic equilibria established between carbon dioxide and carbonate/bicarbonates of calcium and magnesium determines the solubility of CO_2. To reduce errors associated with CO_2 solubility, large increase of pressure should be avoided and can be done by venting the accumulated gas regularly. Another option is through use of a suitable barrier solution such as highly acidic or saline to avoid CO_2 diffusion in the liquid. An alkaline solution is an alternative if only methane needs to be measured since all the carbon dioxide in the off gas will be absorbed.

1.3.4 Summary of anaerobic biodegradability tests

This section has outlined the existing methods for measuring anaerobic biodegradability which builds upon chemical analysis techniques (e.g. COD, TOC, DOC; ^{14}C labeled substrate; Coenzyme F_{420}), microbiological based methods (16S rDNA; DGGE; FISH) and gasometric measurement techniques (volumetric and manometric).As pointed out earlier, these methods differ from each other in a variety of ways. The procedures, materials, detection systems and output differs considerably thus the method of choice is up to the researcher to decide.

However, in the field of environmental technology the gasometric measurement techniques are generally preferred due to the information that is obtainable with the method as well as the availability of laboratory equipment and knowledge. The demands for sophisticated technology and equipment along with requirement of microbiological knowledge with the other analytical methods limit their applicability across environmental engineering research. Based on this logic this research will be focusing on the gasometric approach for measuring anaerobic decomposition, and improvements for the current technique will be recommended.

1.4 ANAEROBIC DIGESTION FEEDSTOCK

1.4.1 Feedstock Potential

The development of anaerobic digestion was primarily to care for liquid wastes with or without suspended solids, such as domestic or industrial wastewaters, manures and sludges from biological or physic-chemical treatments, among others. In the recent years, increased environmental awareness, coupled with the demand for sustainable waste management strategies and renewable energy form, has widened the application for anaerobic digestion. Organic waste diversion from landfills including domestic and industrial organic wastes has gained growing recognition locally and internationally. It is a move that is motivated towards reducing diverse impacts on the environment such as emanating odours, attracting vermin, emitting toxic gases, contaminating groundwater and wasting landfill capacity. Figure 1.6 summarizes the variety of feedstock used in anaerobic digestion system.

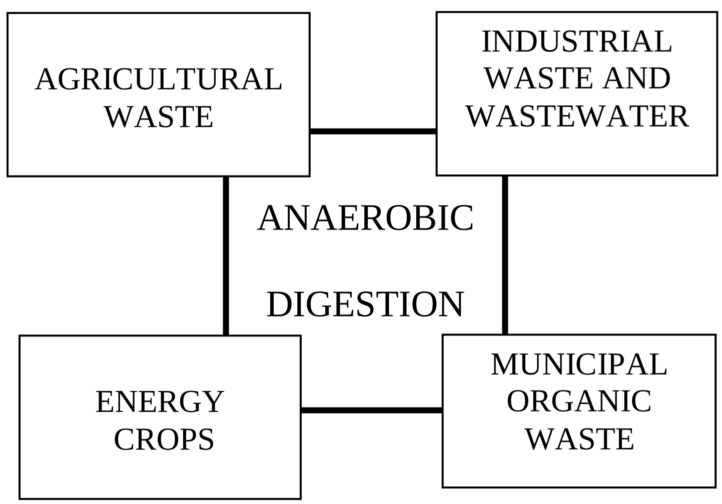


Figure 1.6 Supply of suitable substrates for anaerobic digestion

1.4.1.1 Agricultural waste

Among the agricultural wastes, pig and cow slurry, chicken manure and farmyard manure are of primary concern because of the detrimental effect on the environment. The natural degradation of livestock wastes during storage leads to a release of greenhouse gases (CH_4 and CO_2) to the atmosphere due to the anaerobic decomposition of the organic matter (Vedrenne et al., 2008). In addition, an improper application of animal manure containing nitrogen and phosphorous to land can result in eutrophication of surface waste resources and pollution of soil and groundwater (Gungor-Demirci and Demirer, 2004). Anaerobic digestion has been used in a beneficial way to treat and dispose of the livestock waste by making use of its methane potential. The methane potential of manure comes from the digestion of the organic components in the faeces and in the straw used as bedding material, which is mainly: carbohydrates, proteins and lipids (Moller et al., 2004).

Studies by Gungor-Demirci and Demirer (2004), Kaparaju et al. (2008), Vedrenne et al. (2008), Moller et al. (2004), Kaparaju and Rintala (2008) and Samy (2000) have shown that the by-product of cattle, poultry, and swine are suitable as feedstocks for anaerobic digestion. The methane potential for pig can be expected to be between 0.244 – 0.343 L CH_4/g VS while sows are from 0.260 – 0.334 L CH_4/g VS. The average methane potential from dairy cattle slurries is estimated at 0.243 $\pm$ 0.041 L CH_4/g VS and is usually lower than the average methane potential from swine slurries at 0.297 $\pm$ 0.040 L CH_4/g VS. The methane yield from calves and duck slurries are 0.386 and 0.319 L CH_4/g VS, respectively (Vedrenne et al., 2008).

The methane potential of livestock waste is influenced by the different organic matter compositions related to animals diet (Vedrenne et al., 2008). Cattle are fed with roughage which contains greater amounts of lignin and cellulose than pig feed does. Furthermore, the amounts of proteins and lipids are considerably higher in pig than cattle manure. Due to the higher proportion of lipid in pig manure, theoretically, the methane would be higher in both pig and sow manure than in cattle manure (Moller et al., 2004). The amount and type of bedding material also affects the methane productivity of the livestock waste. The use of straw as bedding material (10 g straw per 1 kg manure) has been found to increase the methane yield of the manure by 10% (Moller et al., 2004).

1.4.1.2 Energy crops

Cultivation of crops specifically for anaerobic digestion purposes could be of interest for countries where energy costs are high and where sufficient agricultural land is available. Maize has been shown to be an excellent energy crop, capable of producing methane between 0.211 L CH_4/g VS (Raposo et al., 2006) to 0.300 L CH_4/g VS (Pereira et al., 2009). Chynoweth et al. (1993) when investigating the methane potential of several biomasses, found methane values were low with crops containing more lignin or the presence of inhibitors (e.g. tannins and resins in soft woods). Methane yield increased with harvest frequency especially for napiergrass, and that methane is generally higher in leaves than in stems. The study by Chynoweth et al. (1993) also found that post-harvest conditions such as ensiling or drying did not have a significant influence on methane yields. Although the production of energy crops for the purpose of producing bio-oil has been proven viable, its full scale adoption is still yet to be well accepted. Continuing argument exists over the importance of land use for human consumption over energy production, especially for areas where arable lands are scarce.

1.4.1.3 Industrial waste and wastewater

Huge amounts of agricultural raw materials like plants and livestock are processed in the food industries. During processing, wastes and wastewater are produced which are typically high in organic matter, making these waste products feasible feedstock for anaerobic digestion. Some examples include wastewater from soybean (Yu et al., 1998) and orange juice (Siles et al., 2007) processing and spent apples from producing apple juice (Frederic et al., 2007). The meat processing industry emits grease trap sludge which are lipid rich, have small fibrous structure and high water content (Luste et al., 2009). Lipids have high methane production potential but when excessive, can be inhibitive due to the production of long chain fatty acids (LCFAs). The digestive tract content comprise of partly digested fodder high in carbohydrate and lignin, which could be resistant to anaerobic breakdown. The effluent generated during the unloading of fish from ships is also high in organic load. The wastewater produced from the fishmeal industry contains elevated sulphide and sodium, however has been shown susceptible for anaerobic treatment provided a suitable adapted inoculum is used (Aspe et al., 1997). One of the drawbacks with most food processing industries as anaerobic digestion feedstock is the seasonal feed variation having an effect on the quality and quantity of gas production. This is because the availability and demand for particular product changes considerably during the year.

1.4.1.4 Municipal organic waste

Anaerobic digestion is an attractive treatment strategy for the municipal solid waste as the waste stream contains a large proportion of organic materials, of about 60%. Of this percentage a high fraction includes waste paper (22%) followed by kitchen organics (16%) and garden trimmings (22%) whereas the remaining constituents are non organics such as plastics, textiles and rubber, rubble, soil, metal, glass, sanitary and hazardous materials (Street and Zydenbos, 2004).

Chanakya et al. (2009) tested the decomposition patterns of vegetables, fruits, fresh leaf litter and paper as feedstock. The vegetable and fruit wastes fermented rapidly in an anaerobic digester, marking their suitability as feedstock. On the other hand, leaf litter and newspaper fermented slowly with poor process stability and moderate biodegradation, achieving only 25-50% decomposition in 30 days. Anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) has been demonstrated to proceed best using digested sludge as inoculum and worst if inoculated with cattle manure, causing a restricted removal of organic matter and methane yield (Forster-Carneiro et al., 2006). The maximal methane generation can be obtained under optimal pH, temperature and initial total solids concentration, which can be predicted using a program developed by Liu et al. (2008) for the decomposition of OFMSW. The municipal organic waste steam has been successfully digested at pilot scale under thermophilic conditions, achieving 80% volatile solids degradation, methane yield of 300-400 Nm^3 CH_4/ton VS at a retention time of 15 days (Davidsson et al., 2007).

1.4.1.5 Co-digestion

Co-digestion can be defined as one of the advantages of the anaerobic technology where several wastes with complementary characteristics are combined in a single treatment (Fernandez et al., 2005). One of the objectives of co-digestion is to improve the methane production of feedstock which otherwise if digested on their own would produce a low methane yield either due to its low biodegradability (e.g. due to lignin) or presence of inhibitory compounds like potassium and lipids. Fresh vegetable waste digested alone did not produce methane due to their high potassium contents (55 kg/kg dry weight fresh vegetable waste). Adding (40% on wet basis) sludge from an agro-industrial wastewater to the fresh vegetable waste (60%) improved the rate and yield of methane production due to the dilution and synergic effects (Carucci et al., 2005). Lipids (fats, oils and greases) can be found in most food waste and industrial wastewaters such as slaughterhouses, dairy industries or fat refineries. The digestion of food waste and cow manure has been shown to improve by the addition of oily wastes (Neves et al., 2009). The co-digestion with fat increases the amount of biogas produced according to the organic loading imposed (Fernandez et al., 2005). Fat from animal origin (94% degraded) performs no different to fat of vegetable origin (97% degraded), which is of special interest in the industrial application of co-digestion process, where the co-substrates may be variable.

Another basis for co-digestion is when seasonal variations could be affecting the supply of anaerobic digestion feedstock, especially the case for agricultural waste and industrial by-products. Carballa et al. (2007) found that vegetables like cabbage, celery, tomato and potato can be co-digested indicating the possibility of treating different fruit and vegetable wastes together in one plant. The highest methane potential of 465 L CH_4/kg VS was achieved for a combination of tomato, potato and cabbage while the least was observed with tomato, potato and celery mixture, producing just 235 L CH_4/kg VS.

1.4.2 Issues with the feedstock

When designing for anaerobic digesters to be operating on solid waste feedstocks, the physical and chemical characteristics of the waste require careful consideration since they affect the biogas production and process stability during anaerobic digestion (Zhang et al., 2007). The biodegradability of the waste, its moisture and volatile solids content, the presence of toxins, nutrients and fiber contents as well as the variability and particle size of the feedstock are some of the characteristics that may exhibit a profound effect.

For food wastes, scraps of fruits, dairy products, honey, and maple syrup contain large amount of simple carbohydrates and are the first ones to break down. Complex carbohydrates occur in wheat products (such as bread and pastas), other grains (such as rye and corn), beans, and root vegetables (such as potatoes). Meat, poultry and fish are protein rich. Because protein consists of amino acids that are strung together in complex formations, they require more time to break down. The study by Lay et al. (1997) confirmed that carbohydrates are more degradable than lipids and proteins. Methane fermentation from carbohydrate was more rapid especially when leachate was reintroduced into the reactor. Pichler and Kogel-Knabner (2000) found that when treated anaerobically, carbohydrates experienced the highest mass loss compared to protein and lipid. The latter was influenced by microbial resynthesis and recalcitrance.

There are some common substances that can affect the anaerobic digestion process, considered as toxins especially if present at excessive levels. VFAs, pH, free ammonia and hydrogen sulphur are the most frequent (Mata-Alvarez, 2003). pH has a definite effect on the threshold levels of VFA, ammonia and hydrogen sulphide (H_2S) in which for these substances, the toxic species is the undissociated one. Lipid rich substrates are at risk of experiencing anaerobic treatment problems. This is because lipids adsorption onto biomass can cause sludge flotation and washout. In addition, lipids contain LCFAs where in high concentrations can inhibit methanogenic bacteria due to damage to cellular membrane (Fernandez et al., 2005). As mentioned earlier lipids can be found in most food and livestock waste having a high fat, grease or oil content.

Papers are cellulosic materials and are low in nitrogen. Green waste can vary in its degradability: grass clippings have high nitrogen content and are readily degradable, (Haug, 1993) leaves typically are nitrogen poor but are otherwise degradable, while woody materials are more slow to degrade. Garden wastes are indeed known to yield much less biogas, relative to kitchen wastes, due to the higher proportion of poorly degradable lignocellulosic fibres (Mata-Alvarez, 2003). Chandler et al. (1980) observed a strong inverse correlation between volatile solids (VS) destruction and initial lignin content and thus concluded that lignin was the predominant factor to determine the extent of organic substrate degradation in anaerobic conditions. Ligno-cellulosic content was a reliable anaerobic degradation predictor as well where biodegradability decreases with the increase of the waste ligno-cellulosic content (Buffiere et al., 2006).

The municipal solid waste stream may vary considerably with climate, season, disposal practices, and a wide range of socioeconomic factors (Tchobanoglous et al., 1993). Take food waste for instance, the amount being wasted domestically varied according to an individual's diet and food preferences such as:

(i) Avoidance of meat by vegetarians;

(ii) Eating habits such as compulsive eating or being health conscious;

(iii) Certain religious requirements;

(iv) The household's composition or income.

A family with two preschool children, a house with four university students or a flat with two elderly people would consume and discard different types of food. In addition to the municipal source, there are also a wide range of other organic feedstock sources. This includes the institutional sources like the hospital kitchens, commercial sources comprising of restaurants and markets as well as industries for example food processing.

The extent of recycling, collection frequency as well as changes in technology also affects the quality of the municipal solid waste. The biodegradation kinetics has been reported to be 5-10 times larger for source-sorted municipal solid waste compared to mechanically-sorted ones (Mata-Alvarez et al., 1990). When waste was sorted at the source, a maximum kinetic constant, k of 3.53 d^{-1} is reported whereby 406 L CH_4/kg VS is produced and achieving 98% biodegradation. A lower kinetic constant of 0.43 d^{-1} was obtained when digesting waste that were mechanically sorted resulting in a lower methane generation and substrate biodegradation of 131 L CH_4/kg VS and 87%, respectively. The higher biodegradation and methane yield with source-sorted waste were due to absence of non-biodegradable portions namely large amounts of suspended solids, small pieces of plastic, wood, paper, etc. which were otherwise found with the mechanically-sorted MSW.

The variation in waste generation especially its composition and quantity as well as the collection regime changes the setting for microbial growth within the rubbish pile. These factors and others created the non-homogenous nature of the municipal solid waste. The heterogeneity of the solid waste poses challenges for the researcher intending to conduct analysis on the waste.

1.5 RESEARCH NEED

One of the single most relevant issues with the commercial adoption of anaerobic digestion will always be cost, mainly in respect to the capital and operating costs. In addition, this is further risked with the susceptibility for anaerobic systems to failures being easily affected by the complex mixtures being treated, toxic materials, improper design or operation. Operating and maintaining healthy anaerobic digesters also requires understanding of the substrate biodegradability, gas yields, digestate quality along with forecasting other anaerobic problems. As such, plant designers often resort to laboratory scale investigations first, carefully avoiding a greater overall economic risk.

As was discussed, of the various laboratory methods available for the above purpose, the gasometric means are currently the preferred options in the area of environmental engineering. Here, the continuous procedures closely simulate a full-scale anaerobic operation. The batch tests on the other hand, can produce comparable results within 20-60 days and demands less apparatus, labour, services and time. The batch test typically used to assess anaerobic biodegradability of liquid samples involves substrates being incubated along with seed, nutrient and buffers in 250 ml to 5 L bottles (Owen et al., 1979; Bogner, 1990; and Wang et al., 1994). The methane contribution resulting from sample decomposition is obtained by subtracting the background values (seed blanks) from the total.

These batch tests are of little value for many solid samples. One concern is the need to modify the solid sample prior to testing. Most test methods involve drying, grinding, re-drying and re-grinding to 2 mm or less. These modifications make the test results difficult to apply to field conditions. In addition, the steps involved in sample preparation could lead to oxygen exposure, which could distort the results. Because of a small sample size of about 10-50 g w/w the test result may not be representative of the bulk material. Apparently, even though the batch test described above has been accepted as a standard procedure for determining ultimate anaerobic biodegradability, there are still room for further modifications.

Finally, the anaerobic digesters are largely designed from empirically derived information such as hydraulic retention time, organic loading rate etc. Consequently, the digesters are frequently overdesigned and subject to unpredictable failure in performance. The use of comprehensive data from biodegradation yields and kinetics should lead to more economic design and predictable stable performance (Mata-Alvarez, 2003 pp 56).

1.6 RESEARCH OBJECTIVE

The first aim of this research is to develop a new tool enabling analysis on larger sample sizes with less sample disruption or interruption. The tool would be applicable to a range of substrates of varied degradability such as kitchen leftovers, rice, starch, sugar etc.
While using this tool, the following objectives will be pursued:

(i) To suggest a suitable operating procedure for the developed tool including mixing, nutrients or buffer supplementation, substrate loading and others. The reproducibility and repeatability of the device will also be validated.

(ii) To determine anaerobic biodegradation kinetics of the substrate and establishing correlations (if any) of the device to the smaller scale batch tests (biochemical methane potential, BMP) and the bench scale continuous reactors.

The respirometric device proposed in this research is purposely well sized so that it can cater to a variety of solid sample, sizes and conditions. Moreover, investigations regarding sample pre-treatments such as size reduction, blended, grinded or mashed can be made possible. It is anticipated that this device would open up interesting findings not before attainable using previous methods. The upgrading of the current technique of determining anaerobic degradation is valuable since batch bioassays are being used as initial screening tests when planning for large scale waste treatment plants.

1.7 THESIS LAYOUT

This thesis consists of 7 seven chapters. In the first part, the thesis introduces the anaerobic digestion process as a suitable treatment option for organic solid waste. Here, a brief review of available methods to measure anaerobic biodegradation is given. This introduction is necessary to provide a thorough picture of the anaerobic digestion process as well as the relevance and importance of the preliminary laboratory investigation as a first step in designing a treatment plant. The introductory information also serves to better understand the principals and arguments later in the second part, as well as the origin of weaknesses and issues that was identified, being the objective of this research.

Anaerobic digestion of food waste is used as a case study in many chapters throughout this thesis. Chapter 2 reviews the methods for analyzing the methane potential of food waste from batch to continuous laboratory testing systems to any available commercial apparatus. The aim here is to identify issues of running the tests as well as factors that could affect the result precision and reproducibility. In order to make full use of the small scale test's potential, knowledge of process kinetics and biodegradation yields will also be explored paying special interest to kinetics utilization in process scale up. This review does not attempt to exhaustively describe the literature, but rather to gather the recent publications so as to identify the research status and needs.

A general overview of methodologies used throughout the research is described in Chapter 3, with a more detailed description included in the respective chapters. The equipment, measurement systems and data analysis tools will be presented here. Chapter 4 and 6 are results chapters each focusing on different objectives of the research. The device design and development is detailed in Chapter 4. The design is later validated through several substrate testings mainly food waste and a few rice, carbohydrates standards and glycerol. Chapter 6 focuses on kinetics of food wastes digested anaerobically in the developed respirometric device, the BMP tests and continuous tests. The operation and maintenance of continuous reactor is presented in Chapter 5. The aim here is to identify any relationships between the different digester operating systems. Both chapters begin with the introduction, a brief literature review narrowing the anticipated outcome, methodology relevant to the intended study before presenting the results and discussion.

Lastly, Chapter 7 presents the conclusion derived from the investigations and check whether the objectives of the research was achieved. The emphasis here is to relate findings from Chapter 4, 5 and 6 that is necessary to facilitate the transfer of data found in laboratory to full-scale process, which hopefully would economise the reactor design. In addition, recommendations for further study and suggestions for improvements will be presented.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The anaerobic biodegradability of a substrate can be evaluated either through following the substrate depletion or the production of biogas. In some cases, both parameters are measured and serve to complement each other in order to illustrate a bigger picture of the ongoing anaerobic process. These parameters; the substrate depletion and/or biogas production are monitored by incubating the test substrate under anaerobic conditions at a set temperature under a continuous or a batch system. Both of these systems have already been discussed in Chapter 1.

The interest of this Chapter is to review the available literature, to gather and argue the information pertaining to batch anaerobic systems used to measure the methane potential of mainly organic solid substrates. The literature is compiled in the first part of this chapter. Methane potential of wastes is defined as the ultimate specific methane production, for indefinite degradation time (Angelidaki and Sanders, 2004). In practice the degradation time is definite and the methane potential is estimated by extrapolation of a methane time degradation curve. Methane potential is typically expressed specifically per amount of waste, volume of waste, per mass volatile solids (VS) added or chemical oxygen demand (COD) added. The volume is usually expressed in standard pressure (1 atm) and temperature (0°C) conditions (STP conditions).

Although the methane potential determined in the batch test gives a rough idea of the quality of waste and the potential biogas production, the practical yield obtained in a large scale biogas reactor will always be lower due to a number of factors. According to Angelidaki and Sanders (2004) these are because:

- a fraction of the substrate is utilized to synthesize bacterial mass, typically 5-10% of the organic matter degraded,
- at a finite retention time a fraction of the organic matter material will be lost in the effluent, typically 10%,
- lignin is not degraded anaerobically,
- often a part of the organic material is inaccessible due to binding in particles or structural organic matter, and
- limitation of other nutrient factors.

It would be interesting to see if researchers have investigated the kinetics behind the different digestion systems (batch and continuous) and whether an explanation can be given for the varied methane production between the two. Using this focus, the second part of this chapter hopes to understand the function of kinetics at explaining the anaerobic degradation process. The review also looks at the transfer of data gained from the batch methane potential test to the biodegradation kinetics and how this information can be used when designing or operating a continuous reactor (CSTR) system. This is important as large scale digesters are mostly performed in a continuous mode.

Considering the vast number of kinetic models and their modifications in the literature, in most part, only the first order kinetics is reviewed in this chapter. As this thesis is not a modelling exercise, rather, a research that was initiated to develop a methodology to measure anaerobic decomposition of solid waste, it is felt unnecessary to consider more complex kinetics. Should the former would have been the intention, a more thorough review of available kinetics would have been carried out and the corresponding experimental and data collection planned.

PART 1: METHANE POTENTIAL DETERMINATION

2.2 AVAILABLE TEST METHODS

2.2.1 Biochemical methane potential (BMP) test

Owen et al. (1979) developed the BMP test by combining the theory and procedures of the anaerobic Warburg with serum-bottle techniques for cultivation of anaerobes. The Warburg apparatus (example shown in Figure 2.1) is an analytical instrument for measuring the pressure of gases and vapors from biochemical reactions. The Warburg apparatus is based on the principle that, at constant temperature and gas volume, any changes in the amount of gas can be measured by changes in its pressure. It consists of a detachable flask for placing the sample which is equipped with one or more sidearms for additions of chemicals and a manometer containing a liquid of known density.

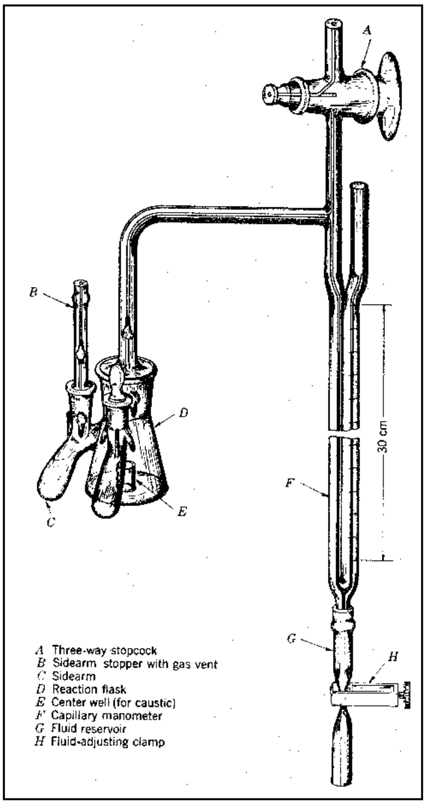


Figure 2.1 The Warburg apparatus (source: Ridge and Seif, 1998)

BMP test was defined as a measure of substrate biodegradability determined by monitoring cumulative methane production from a sample which is anaerobically incubated in a chemically defined medium. The set-up includes 250 ml reagent bottles and rubber serum caps, gassed with a mixture of 30% carbon dioxide (CO_2) and 70% nitrogen (N_2) for 15 minutes, then stoppered and equilibrated at incubation temperature. This was done before introducing samples, defined media and inocula (refer Figure 2.2). Respective gas productions were monitored volumetrically using the syringe method of Nottingham and Hungate (1969). The method comprises a needle which is attached to a 10 ml syringe being inserted into the test vessel. The volume of gas forced into the syringe as the needle penetrates the stopper is noted. The methane contribution resulting from sample decomposition was determined by subtracting background values, obtained from seed blanks, from the sample totals. BMP is referenced to either the sample volume (m^3 CH_4/m^3 sample), sample mass (m^3 CH_4/kg sample) or sample organic content (m^3 CH_4/kg COD).

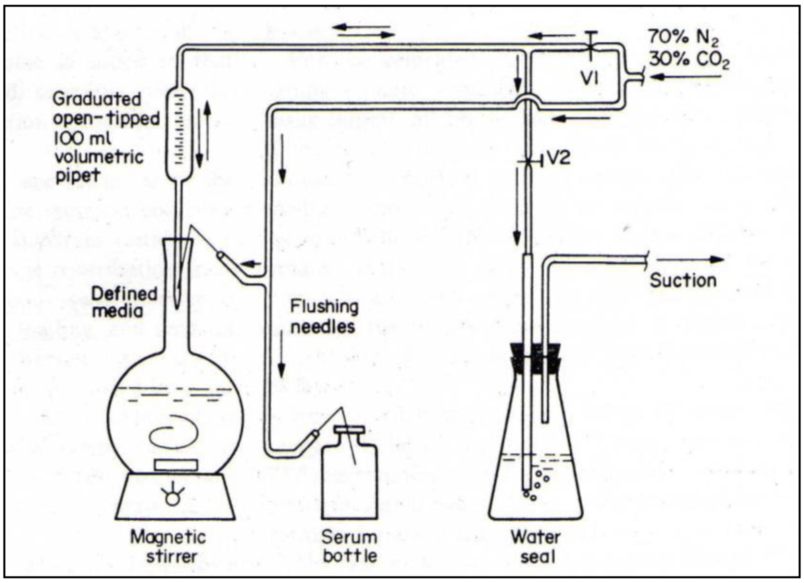


Figure 2.2 Schematic diagram of procedure for the anaerobic transfer of defined media into serum bottles (source: Owen et al., 1979)

2.2.2 Unspecified batch test

Unspecified batch tests are defined here as methane potential determination tests that have not been specifically referred to as a BMP test. Hence, it is not clear if these researchers have referred to the BMP test as a guideline or not when applying their own batch methane potential tests.

The work by Brummeler and Koster (1989) was one of the early applications of a batch reactor system for testing the anaerobic decomposition of the organic fraction of municipal (OFMSW) solid waste. The batch reactors were filled with a mixture of organic fraction, seed, tap water and buffer, flushed with nitrogen gas for two minutes before they were closed and incubated at 30°C. The buffers were mixed with the sample before seed was added to avoid pH higher than 9. The sample which was sourced from a separation plant for municipal solid waste (MSW) was sieved to 12 mm for methane potential testing. No nutrients were supplemented. One of the significant suggestions from the Brummeler and Koster (1989) study was the incorporation of smaller scale identical reactors that were set-up solely for sample analysis (refer Figure 2.3). The idea was developed since it appeared impossible to take samples from the same reactor without significant disturbance of the process. The set-up includes the main batch 6 L reactor which was connected to gas measurement systems to monitor biogas production. The gas measurement system consists of a water lock and a gas tight bag of 10 L for biogas volume determination. A septum is also fixed between the reactor and the water lock device for biogas sampling using a glass syringe for composition analysis on the gas chromatograph. At the same time, five small 0.5 L reactors were filled under the same conditions as the main 6 L reactor. The effluent pH and organic acids were measured from these bottles. Potential methane yield was reported in L CH_4 at STP/kg of organic fraction.

The small batch set-ups for sample analysis idea soon caught on. Rao et al. (2000) and Neves et al. (2008) too set aside some of the batch reactors for content monitoring over time. In the study by Rao et al. (2000), two of the four identical batch reactors fed on food waste were left undisturbed for biogas production measurement while the remaining two were used for sampling and analysis of pH, volatile fatty acid (VFA), COD and alkalinity. The liquid samples may require centrifugation and filtration to 0.2μm for soluble COD and VFA analysis (Neves et al., 2008).

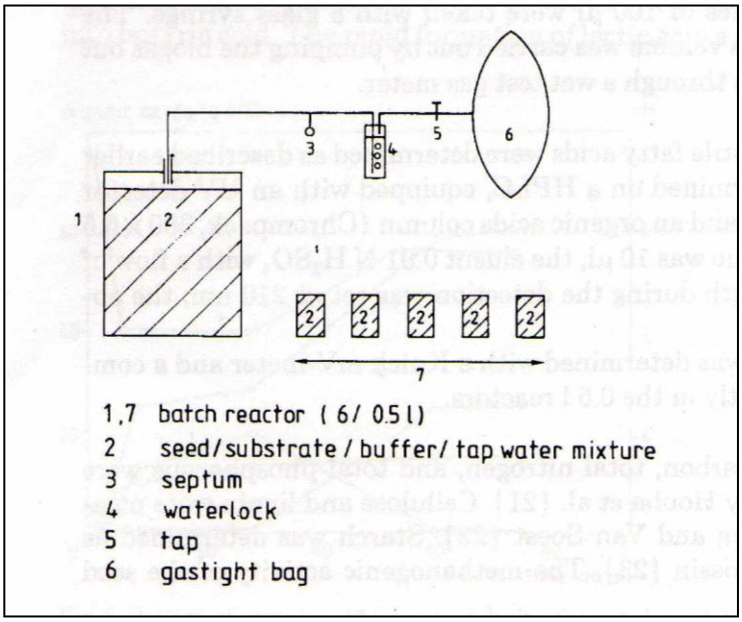


Figure 2.3 Experimental set-up for the dry digestion experiments of the (OFMSW) (source: Brummeler and Koster, 1989)

2.2.3 Gas measurement methods for batch anaerobic tests

The OxiTop® Control system is a manometric device consisting of several bottles placed in an incubator at 35°C, mixed by a magnetic stirrer (refer Figure 2.4). The uniqueness of the OxiTop is its measuring top. Each top registers the pressure in the vessel headspace and stores the data during the whole duration of the trial. Two side-mounted connections which are sealed with septa, are used for substrate injections and for biogas discharge, respectively. When anaerobic degradation has taken place the dissolved CO_2 can be driven off and then removed from the gas space by means of a CO_2 absorber (OxiTop manual). According to the manual, the resulting pressure difference is proportional to the CO_2 concentration; the remaining overpressure is proportional to the methane concentration.

Alternatively, the biogas volume and composition can be determined separately (Caffaz et al., 2007). The cumulative biogas volume is established from the overpressure due to biogas accumulation in the headspace, which is automatically registered by the measuring heads.

The gas composition is analyzed by the gas chromatograph. The initial headspace pressure is set at the atmospheric value, by discharging the overpressure into a water bottle.

Figure 2.4 OxiTop Control AN6/AN12 for determination of anaerobic degradation processes

2.2.4 Challenge AER-200 Respirometer

The Challenge AER-200 Respirometer is another example of a commercial tool to determine methane potential. The AER-200 Respirometer was designed to cater for both aerobic and anaerobic wastewater applications. In the study of Caffaz et al. (2007), the Challenge AER-200 Respirometer consisted of 8 biological reaction vessels (400 ml), a multiple stirring plate and cells containing oil for measuring the evolved biogas. Mineral salts medium, buffer and tap water was sometimes added as per the work of Schmit and Ellis (2001) in addition to other compulsory items like the substrate and seed before flushing the vessel with nitrogen gas to rid of oxygen.

The flow measuring cell is the heart of the AER-200 Respirometer system. During anaerobic tests, the biogas leaves the vessel through Teflon airtight valves, sealed with neoprene bungs and flows through the measuring cell where bubbles of fixed volume are formed. A detection section counts and registers the number of bubbles evolved which are then converted into the corresponding biogas cumulative volume and flow rate. In some tests, a carbon dioxide (CO_2)

scrubber was utilised in order to absorb the carbon dioxide in the gas stream, thus only the methane produced is measured. Logan et al. (2002) replaced the oil in the respirometer with 1 M NaOH while Schmit and Ellis (2001) positioned 50/50 w/w potassium hydroxide-silica mixture in-line before the flow cell.

2.2.5 Anaerobic Biogasification Potential (ABP)

Schievano et al. (2008) referred to the Anaerobic Biogasification Potential assay as similar to the biochemical methane potential (BMP) test. In serum bottles, sample, inocula and deionized water were added. The bottles were sealed with teflon hermetic caps, flushed with nitrogen atmosphere, and incubated at 37°C, until no further biogas production is detected. The volume of biogas produced is estimated by withdrawing extra-pressure gas with a 60 ml syringe, while the CH_4-CO_2 ratio in the biogas is determined using a gas chromatograph.

2.2.6 Biogas Activity Monitoring (BAM)

A computerized automatic biogas activity monitoring system was developed by Angelidaki et al. (1998) for monitoring the production of biogas in closed vessels (refer Figure 2.5). The test was performed in 58 ml serum vials containing 20 ml seed sourced from an Upflow Anaerobic Sludge Blanket (UASB) reactor, buffered with sodium bicarbonate, sealed with butyl rubber stoppers and placed in incubator with stirring. The system consisted of a single pressure transducer which measured the pressure build-up through a motor driven multiport sampling valve in a series of up to 16 test vessels. A standard PC computer equipped with an I/O card positions the multiport valve via an interface power circuit. The gas production of each vial was registered either in a differential or cumulative form in a data file suitable for later numeric/graphic processing with standard spreadsheet software.

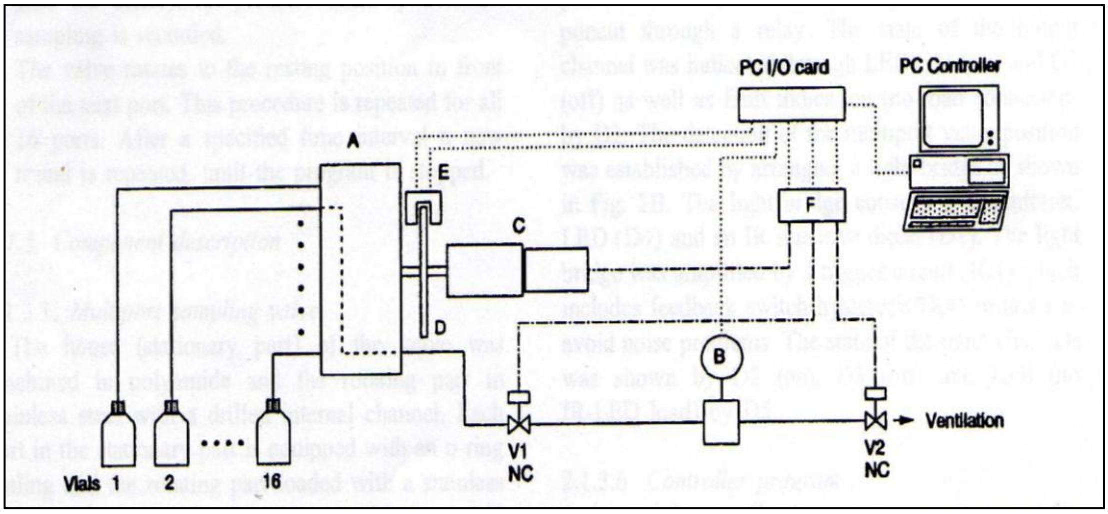


Figure 2.5 Schematic description of the set-up of the BAM system. Multiport sampling port (A); pressure transducer (B); gear and motor (C); position coded plate (D); pickup (E); power interface (F). Valves (V1 and V2) (source: Angelidaki et al., 1998)

2.2.7 Summary

Table 2.1 summarises the applicability of the batch methane potential tests reviewed above across various solid organic samples. It is the purpose of the summary to highlight the many ways these tests have been performed. This includes the different vessel size used, the sample condition, nutrient, buffer and seed addition as well as the gas measuring system.

There appears to be no certain trend over the years indicating the avoidance or the increasing use of a particular material. The choice of the way to conduct the methane potential test is probably more subjective depending on the preference and availability of materials to the researcher.

Table 2.1 Batch test set-up

Waste	Vessel size (L)	Max. Particle size (mm)	Nutrient	Buffer	Seed	Gas measuring method	Test method	References
Organic waste	0.12	2	Yes	No	Lab grown	Syringe	BMP	Lay et al. (1997)
FVSW	0.14	2	Yes	No	Lab grown	Syringe	BMP	Gunaseelan (2004)
Food waste	0.50	4	No	No	Lab grown	Syringe	BMP	Heo et al. (2004)
Solid waste	0.64	3	Yes	Yes	WWTP	Syringe	BMP	Jeon et al. (2007)
Landfill waste	0.50	1.5	Yes	No	Anaerobic sludge	Water displacement	BMP	Bilgili et al. (2009)
Food waste	1.00	n/a	No	No	WWTP	Water displacement	Batch test	Zhang et al. (2007)
Restaurant waste	0.16*	3	Yes	No	UASB plant	Pressure transducer	Batch test	Neves et al. (2008)
Solid waste	0.35*	10	Yes	Yes	Anaerobic sludge	n/a	Batch test	Bockreis et al. (2007)
Landfill waste	0.14	5.6	No	No	Food waste biogas plant	Syringe	Batch test	Tojo et al. (2007)
OFMSW	0.10	25	No	No	Lab grown	Water displacement	Batch test	Charles et al. (2009)
OFMSW	1.14	n/a	No	Yes	Lab grown	Pressure transducer	OxiTop	Caffaz et al. (2007)

Note: *working volume is reported, FVSW: fruit vegetable solid waste, OFMSW: organic fraction municipal solid waste, WWTP: wastewater treatment plant, n/a: not available

2.3 STANDARDISATION OF METHANE POTENTIAL DETERMINATION

A number of studies and research activities dealing with the determination of the biogas potential of solid organic substrates have been carried out in recent years. As a consequence, many efforts have been made to define a protocol for the ultimate methane potential for a given solid substrate. This includes standardisation by the International Organization of Standardisation, the European Chemical Industry Ecology and Toxicology Centre and HMSO (Her Majesty's Stationery Office) UK.

All the standards except for ISO 15473 (2002) describe the determination of gas production using a pressure transducer technique. The method measures the increase in headspace pressure in the test vessels resulting from the production of carbon dioxide and methane (CH_4). ISO 11734 (1995) strongly recommends taking intermediate readings of the gas pressure. By making regular measurements, the kinetics of degradation can be followed. Moreover, the pressure increase provides guidance to as when the test may be terminated.

The ECETOC (1988) and HMSO (1988) specifically mentioned their guideline was developed based on the methods implemented by Shelton and Tiedje (1984). The standards apart from ISO 15473 (2002) describe a similar apparatus and measuring system, the reagents as well as the test procedure. This includes the use of some sort of serum bottle (e.g. Wheaton bottles), butyl rubber serum caps and may include the use of crimped aluminium rings as the test reactors. The re-sealing ability of a septum, after puncturing, can be extended by a blob of silicone rubber sealing compound (e.g. Silcoset 151) on its top (HMSO, 1988). ECETOC (1988) directs that volume of aqueous phase should not be less than 100 ml and the headspace above the liquor should be between 10 and 40% of the total digester volume. Temperature should be maintained at 35°C, using a suitable thermostatically controlled bath or incubator. Reagents include distilled water, medium, trace elements, test compound and inoculum. The most appropriate source of inocula is sludge from the anaerobic digester of a sewage treatment plant treating predominantly domestic sewage. However, a laboratory grown seed is also appropriate. To reduce the background gas production, sludge needs to be pre-digested first (ECETOC, 1988). The reactor contents need to be mixed at least two or three times per week. This can be done by manually shaking the vessels for a few minutes and before each pressure measurement (ISO 11734, 1995).

HMSO (1988) considers degradation is complete if more than 80% of theoretical gas production is produced. On the other hand, the substrate is not degraded if less than 30% of the theoretical gas production is produced and inhibitory if negative cumulative net gas production is obtained. ASTM E2170-01 (2008) suggests that a substrate is most likely to be biodegradable in waste treatment plant anaerobic digesters and in natural anaerobic environment if a high biodegradability result is obtained. On the other hand, a low biodegradation result may not necessarily mean the substrate is anaerobic digestion (AD) poor but instead, other factors could be at play.

The kinetics of degradation are influenced by the nature of the substrate, the quality of inoculum and the ingress of oxygen in the experimental set up (ECETOC, 1988). Oxygen (O_2) contamination can inhibit obligately anaerobic methane–producing bacteria, yet a small amount of oxygen may be tolerated by the system. The aerobic respiration by faculatively anaerobic bacteria in sludge will also result in substrate being utilised for O_2 consumption and not CH_4 + CO_2 production. This will reduce the amount of gas produced in the test (HMSO). Oxygen contamination can be checked by observing the vessels after incubation for 24 h to 48 h where vessels showing a distinct pink discoloration in the supernatant liquid should be discarded (ISO 11734, 1995). The resazurin (which was added earlier in the medium) would change colour to pink indicating the presence of oxygen. ASTM E2170 (2008) stated that toxicity usually can be detected following a lower gas production from the test vessels compared to the blanks. Factors include the conditions in the test may be unsuitable for the growth of an acclimated population or the microbial inoculums may have been inhibited by the test substrate at the concentrations tested. Further testing is advised to assess the anaerobic biodegradation of the substrate.

HMSO (1988) also reported other sources of error and reproducibility. Acidic chemicals may result in a flux of CO_2 into the headspace, while sulphate reducing chemicals can outcompete methanogenic bacteria for acetate and hydrogen (H_2). Sulphate reducing bacteria are widely distributed in sewage and in sewage sludge. ISO 11734 (1995) is also concerned about the carbon dioxide dissolution into the water for a pressurised system like this, which can affect the test result. It is recommended that the concentration of inorganic carbon in the supernatant is taken into account when calculating the total gasified carbon.

The ISO 15473 (2002) provides guidance on testing the biodegradation of organic chemicals in soil samples under anaerobic conditions. The test method differs to the other standards described above in that no seed or medium is included in the test vessel. The soil is tested only with a buffer solution. On top of that degradation is monitored using ^{14}C labelled substances where $^{14}CO_2$ production and $^{14}CH_4$ production are evaluated over time. This is unlike the HMSO (1988), ECETOC (1988) and ISO 11734 (1995) where gas production represents the progress of substrate degradation. At least 5 sampling points in time are advised to establish a degradation curve. This follows the observation that materials degrade rapidly during the early stages of incubation, hence, the following sampling frequency is recommended: 0,2,4,8,16,40 and 100 days after application of the test substance (ISO 15473, 1995). The anaerobic conditions can be checked as suitable if the redox potential is less than 200 mV.

Other examples of standard methods include the ASTM D5210-92 (1992) and the ASTM E1196-92 (1992). The former specifies the determination of the anaerobic biodegradation of synthetic plastic materials on exposure to anaerobic-digester municipal sewage sludge from a wastewater plant, under laboratory conditions. The latter contains guideline that is quite identical to the ASTM E2170 (2008) and has been withdrawn in 1998. The procedure designed in ASTM E1196-92 (1992) was applicable to all organic compounds and describes laboratory protocols for determining the conversion of organic substrates into methane and carbon dioxide. Due to the ASTM E2170 (2008) describing known information regarding apparatus, inoculums, test procedure, and data reporting that is reasonably similar to the other standards (HMSO, ECETOC, ISO 11734, and ISO 15473) that has been described here, it is decided that further repetition is not necessary. It is in the author’s opinion that it would be more beneficial for a reader to gain further information from ASTM E2170 (2008) directly.

The most recent effort on standardisation of the batch methane potential test was initiated by the Task Group for the Anaerobic Biodegradation, Activity and Inhibition of the Anaerobic Digestion Specialist Group of the International Water Association (Angelidaki et al., 2009). The report by the Task Group differs to the above standards and guidelines in that a more detailed definition is given for the substrate, inoculums, particle size of the substrate, inoculum activity, medium, mixing, blanks and controls. The definition gave information on what can be expected under certain conditions, and what should be avoided which could consequence in a limited methane production. However, no specific concentrations, volumes, size or thresholds values were specified for each of these items. The report is also the first to offer guideline relating the experimental data obtained from a batch test to biodegradation kinetics. The first order hydrolysis model was recommended for this purpose. As there is quite an exhaustive information, mostly in the quantitative form, it is felt more appropriate to just include a vague description of the report as above in this review. Interested readers could easily obtain and understand the paper by Angelidaki et al. (2009) as the writing is clear and concise.

2.4 FACTORS AFFECTING METHANE POTENTIAL TESTS

The above review of the available methane potential tests and the standard protocols confirms that efforts to quantify anaerobic decomposition through bioassay have been made as early as the 1970s. From then on various refinements have been made aimed at improving and simplifying the preceding methods and to suit the sample tested. The BMP assay by Owen et al. (1979), various other batch test systems, the ABP, BAM, OxiTop and Challenge respirometer systems all conclusively employ some type of serum bottles in which gas production was measured from the sample incubated with anaerobic seed. Methane production of the sample is obtained through gas measured via pressure difference, volume of liquid displaced or bubbles formed, and/or analysis of the gas composition, and after methane coming from the seed is deducted. Although the underlying technique is similar, the methane potential tests between various research applications varied in terms of the seed used, sample pre-treatment and size, nutrient, buffer, reactor type and size, incubation time and temperature, mixing and others. The different configurations and its implications to the methane potential determination are discussed here.

2.4.1 Equipment

As earlier mentioned, the degradation of a substrate can be assayed using either a batch or a continuous flow mode. Most tests however are performed in batch conditions, which normally require simpler equipment and enable the kinetic data to be obtained in a shorter time.

A large stirred batch reactor that can be manually or automatically sampled during the degradation process may be used. It is best used when the degree of degradation is determined via the continuous measurements of the gas production and/or of dissolved products in the reactor mixture. It might be less suitable for the direct measurement of suspended COD or individual particulate components, as possible floating or settling layers and lipids attached to the reactor wall make representative sampling difficult. If this is the case, a system comprised of several small reactors is more suited: at the start of the experiment, all flasks have the same content and it is assumed that the progress of the degradation is similar in all units. Instead of sampling from a single reactor, some flasks are periodically sacrificed and their content analysed. This procedure allows thorough homogenization prior to sampling and the empty flask can be rinsed with an organic solvent to remove particles or lipids attached to the wall.

2.4.2 Alkalinity

Alkalinity is a measure of the buffering or acid-neutralizing capacity of the digester. The pH of anaerobic digestion (AD) is a function of the volatile fatty acid, carbon dioxide pressure of the gas, as well as the alkalinity. The optimum pH for anaerobic digestion is normally in the range of 7-8, pH levels that deviate significantly from this range can indicate potential toxicity and digester failure. Low pH levels, for example, can be a symptom of digester imbalance. As volatile acids concentrations increase, the pH in the digester decreases. As pH levels fall below 6.0-6.5, the acidic conditions produced become increasingly toxic to methane bacteria.

Depending on the stability of the anaerobic digestion process and its potential for producing alkalinity, means for increasing alkalinity and pH may be needed. To increase alkalinity and control pH in laboratory-scale digesters, sodium bicarbonate (Na_2CO_3) is frequently added either in place of or in addition to sodium hydroxide (NaOH). Added buffers from as low as 1500 mg/l Na_2CO_3 (Soto et al., 1993), increasing to 2500 mg/l sodium sulphide (Owen et al., 1979) and up to 9100 mg/l sodium bicarbonate (Raposo et al., 2006) have been added to the BMP assay to prevent acidification of the reactors at its early stage. Alkalinity may also be increased by adding lime, or it may be generated through protein degradation to ammonia (NH_3), which can combine with the carbonic acid in solution to form an ammonium bicarbonate buffer.

Biey et al. (2003) found that if the pH is corrected from the beginning, the biogas production from vegetable fruit garden waste can be shortened to less than 2 months. When substantial buffering was added to the reactors at the beginning, the pH of the digester was maintained above 7.0 and within the optimal range for methanogenic activity (Raposo et al., 2006). The pH stability was attributed to the high buffering capacity of the reactors with an initial total alkalinity of 9100 mg/l. Even when the total alkalinity dropped to lowest of 8000 mg/l, alkalinity was gradually restored and stabilised at 9400 mg/l.

Although the buffer addition may help maintain neutral conditions within the reactor and prevent the accumulation of acid conditions which could lead to reactor failure, excessive addition can have a detrimental effect. Researchers have shown that excessive sodium concentrations can be toxic to the digestion process and should be avoided. The addition of sodium hydroxide as a pH adjustment chemical in the work of Ngian et al. (1978) produced a higher volume and faster rate of gas from pig faeces at 7 and 9 g NaOH/100 g DM. However, both the volume and the rate of gas production decreased significantly at 9 and 12 g

NaOH/100 g DM sodium hydroxide treatments, prompting a sodium toxicity threshold of 0.2 M (12 g NaOH/100 g DM) to be proposed. Kuo and Cheng suggested the possibility of 50% gas production if more than 7 g/l of sodium is present (Kuo and Cheng, 2007), while Brummeler and Koster (1990) reported sodium inhibiting the methane formation at concentrations exceeding 7 g/l, when a shock load is applied. Inhibition of high calcium ion concentrations is likely to be in the same order of magnitude as sodium ion inhibition, although there are indications that the former is slightly less toxic to methanogens. Calcium ion, potassium ion and magnesium ion did not cause significant inhibition at 149–723 mg/l, 231– 460 mg/l and 223– 390 mg/l, respectively (Kuo and Cheng, 2007).

It is in the opinion of this research that anaerobic digester operators need to be aware of the benefits and consequences to buffering the anaerobic digester. The amount and type of chemicals used have to be carefully weighed so that an economical and effective choice is made, rather than blindly adding chemicals which are ineffective or worse toxic to the anaerobic process. The key is to realize whether buffering is needed and whether other means e.g. gradual loading/ extra inoculum/ lower loading rate can be applied instead to counteract the effect of the initial acid burst.

2.4.3 Liquid-to-void volume ratio

ISO 11734 (1995) suggested that the headspace volume shall be 10% to 30% of the total volume. If biogas is regularly released, about 10% headspace volume is appropriate but if the gas release happens only at the end of the test, 30% is appropriate. Pagga and Beimborn (1993) observed greater deviations in replicate tests (more than 20%) when small digesters (<150 ml) or headspace volumes greater than 20% of the digester volume were used. Although the report by Pagga and Beimborn suggests the influence of reactor headspace in replicate reproducibility, the substantiality of the report is doubtful due to the lack of supporting results. Only a table summarising the degradation of palmitic acid and polyethyleneglycol 400 with standard deviation values were given. These summarised values were drawn based on an inter-laboratory test from 21 participating laboratories, where unfortunately no specific information on test procedure and degradation result pertaining to each laboratory was given. In light of this shortcoming, it will be valuable to further substantiate the claims by Pagga and Beimborn (1993) regarding effect of reactor headspace on replicate variability.

The concerns raised by the above researchers were probably regarding the risk from overpressure if the pressure build-up in the reactor is not released as required, leaving the danger of reactor explosion. It could also be that pressure build up within the reactor can increase the chances of carbon dioxide dissolution into the liquid which may affect biogas production measurement.

However, it is our belief that these concerns are more of an issue for small scale reactors probably less than 1 L. With small reactors, a large void headspace means that only a small portion of the reactor volume can be used. As a result, the substrate loading would be very small and inundated by a large seed volume. The gas production would be low and this may affect the precision of the test result, especially if a heterogeneous sample is involved.

On the other hand, a larger size reactor e.g. more than 2 L does not merit the same issues. This is because with a large reactor, every aspect and ratio is intensified. Sample loading can be larger as is the seed. Hence, the void to volume ratio becomes less critical especially since the same amount of biogas can be produced for the same sample loading.

2.4.4 Incubation period

Since the cumulative methane production curve only asymptotically approaches the methane yield, a digester would take infinite time to produce 100% of methane potential. Having final biodegradation percentages closer to 100% is therefore a better basis for assessing total methane potential than the time taken to fully degrade a sample. An incubation period of 60 days is recommended by ISO 11734 (1995) and Shelton and Tiedje (1984). This is generally when the maximum degradation has been reached, and indicates a sufficient degree of biodegradation; > 50% for ISO 11734 (1995) or > 75% of the theoretical value for Shelton and Tiedje (1984). The 60 days incubation period serves only as a guide. The test can be finished sooner if the biodegradation curve of cumulative methane production over time has reached a plateau phase. If at the end of the normal incubation period, a plateau phase is obviously not reached, the test should be prolonged until it is reached. Other researchers (Nopharatana et al., 2007; and Koppar and Pullammanappallil, 2008) have used 95% substrate degradation of the theoretical value as the benchmark to indicate the overall degradation time required. A period between 10-14 days was taken by Nopharatana et al. (2007) to achieve 95% of the theoretical value benchmark.

A sufficient incubation period is important to capture the representative anaerobic degradation and methane potential of the sample under examination. Incomplete biodegradation distorts reproducibility of results and is usually the case with substrates which are difficult to degrade. Such results could be due to poor technique, faulty equipment, a relatively inactive seed, or susceptibility of only a portion of the substrate to degrade. This includes consideration of a lag phase in the beginning of the incubation days until significant degradation starts. An occurrence of lag phase can be as short as a few hours to as long as a couple of weeks, thus affecting the period of batch test incubation. A study on the anaerobic degradation of p-cresol and phthalic acid in fresh sludge had a lag time from 2-4 weeks (Shelton and Tiedje, 1984), thus, shorter incubation period is not advisable. A digester lacking in active MSW degrading microorganisms goes through a longer lag phase.

The lag phase can be eliminated through acclimation as was shown by Nopharatana et al. (2007), where acclimation can be made with readily soluble COD of MSW. A reduction in lag time was observed from 1.7 d to 0.6 d in the second spike of soluble substrate. They mentioned that the reduction in lag time indicated acclimation of the organisms in the reactor. However, the third spike saw an increase in lag time to 2.0 days, due to the spiking done 20 days after the second spiking had completely degraded. This incident was attributed to the number or activity of bacteria in the reactor which may have decreased due to the lack of substrate, later confirmed in a specific methanogenic assay (SMA). This was similar to the observation by Clarke et al. (2007) where a prolonged startup period to initiate methanogenic conditions was due to a poor inoculum. Clarke et al. (2007) for example, took six weeks before methane production began in the anaerobic reactor. A lack of H_2 utilising methanogens is evident by the build up of H_2 during the first 3 weeks of digestion.

For this purpose, a suitable incubation period should cater for the degradation period after the lag phase until a plateau phase is reached in the cumulative biodegradation curve. This may be from a period of 20 to more than 60 days depending on the degradability of the substrate. The consideration of a lag phase is of great importance for a correct activity determination.

2.4.5 Incubation temperature

Although microorganisms with potential importance to the anaerobic digestion process can exist under a wide range of temperature conditions, most studies of practical process applications have been performed under ambient (20-25°C), mesophilic (35°C), or thermophilic (55°C) temperature conditions (Chynoweth and Isaacson, 1987). A study by De Baere (2000) found that most anaerobic treatment plants in Europe operated at mesophilic conditions, which was 62% as surveyed in 2000. The choice may have been driven by the high sensitivity of bacteria to temperature fluctuations in the thermophilic range.

Studies have generally demonstrated that higher temperatures promote increased conversion rates but also decrease process stability (Chynoweth and Isaacson, 1987). It is vital for thermophilic operations that the inoculum be adapted to the same temperature level as intended for the process, or at least to seek inoculum adapted to a temperature within $\pm$ 10°C of the new process (Angelidaki et al., 2006).

For the purpose of conducting batch anaerobic degradation tests, the choice of the incubation temperature would probably be dictated by the purpose of conducting the test itself. For example, one would probably use a thermophilic condition if the objective was to test out preliminary conditions for a full scale thermophilic treatment plant. Otherwise, mesophilic temperature could be more suitable due to ease of operation, less heating requirements and enhanced process stability. Regardless of the choice of test temperature, special care has to be given to avoid the occurrence of unstable temperature, as this can lead to anaerobic process imbalance. Anaerobic digestion imbalance can disrupt the metabolic activities of any group of the consortium of bacteria that perform the diverse function of digestion (Chynoweth and Isaacson, 1987), which ultimately produce conditions that become toxic to the digestion process.

2.4.6 Mixing

Reactor mixing is aimed at creating a homogeneous substrate preventing stratification and formation of a surface crust, and ensures solids remain in suspension (Kaparaju et al., 2008). Further, mixing also enables heat transfer, particle size reduction as digestion progresses, and release of produced gas from the digester contents.

There are many ways in which a reactor can be mixed. These include redistribution using pumps, mechanical mixers or by recirculation of digester contents (Karim et al., 2005). Manual mixing has also been performed especially for small scale reactors. Zhang et al. (2007) manually mixed the 1 L batch reactor once a day as was also done by O'Sullivan et al. (2005) for their 2 L batch vessels. The vessels were mixed at sampling times (usually daily) and were settled at all other times. Birch et al. (1989) recommended for an anaerobic degradability batch test that the reactor contents need to be mixed carefully by stirring or shaking for a few minutes at least 2-3 times per week and before pressure measurement to resuspend and ensure gaseous equilibrium.

Mixing strategy, intensity and duration as well as the location of the mixer has been reported to affect digester mixing. In regards to the mixing strategy, the effect of mixing continuously, minimal mixing (10 min before extraction/feeding) and intermittent mixing (continuous mixing but withholding mixing for 2 hours before extraction/feeding) have been compared. One study saw improved biogas production when mixed continuously compared to if no form of mixing was performed (Ho and Tan, 1985). Recently, Kaparaju et al. (2008) demonstrated an opposing result where minimal mixing improved biogas production compared to intermittent or continuous mixing. This observation follows the earlier findings by Chen et al. (1990) and Smith et al. (1996) where intermediate mixing appears to be the most optimal for substrate conversion. The increased methane production under minimally mixed conditions was attributed to a better syntrophic association between H_2 producing and consuming organisms (Kaparaju et al., 2008). This would allow the syntrophs and methanogens to consume fermentation products without any VFA build-up. On top of that, continuous mixing can lead to enhanced diffusion of inhibitors (e.g. VFA) during incubation into methanogenic area which could inhibit the growth of methanogens. Thus, Vedrenne et al. (2008) suggested the exclusion of continuous mixing during the incubation for the determination of ultimate methane yield in a batch test.

Mixing intensity was also shown to affect digester performances and biogas production (Stroot et al., 2001; Vavilin and Angelidaki, 2005). Minimal mixing was found to be sufficient to distribute the feed adequately and stimulate the formation of new initiation centers that are required for autocatalytic reactions (Vavilin and Angelidaki, 2005). On the other hand, when mixed vigorously, a disruption to the structure of microbial flocs was observed. This in turn disturbs the syntrophic relationships between organisms thereby adversely affecting the reactor performance. Under vigorous mixing, a low and delayed methane production was noticed, especially under high substrate to inoculum ratios. This indicates that methanogenesis was inhibited as the produced VFA was distributed homogenouesly in the batch test bottle. By switching from vigorous to gentle mixing conditions, propionate was quickly consumed, while continued vigorous mixing resulted in propionate persistence.

The review here seems to indicate contradictory effects of mixing strategy and intensity on the performance of batch anaerobic digesters. Adequate mixing was shown to improve the distribution of substrates, enzymes and microorganism throughout the digester whereas inadequate mixing was shown to result in stratification and formation of floating layer of solids. It is therefore important for the reactor operator to realise which mixing regime is most suitable for their set of substrate, reactor and operating conditions by conducting preliminary investigations.

2.4.7 Inoculum

A viable seed can be derived from the environments in which the anaerobic methanogenic decomposition of organic compounds occurs naturally, for example, anaerobic sewage digesters, anaerobic lake sediments or from animal faeces. In anaerobic conditions the use of acclimatised organisms eliminated the need for extended incubation periods. However, the test precision and accuracy are functions of the standard error of estimate of the methane contributions due to seed inocula (Owen et al., 1979).

2.4.7.1 Seed source

Because sewage commonly would have more fat and protein and less carbohydrate than food waste one might argue that sewage digestion would not provide the ideal seed source. However, past studies have shown that seed from a presumably more active cellulolytic environment such as the rumen (Chynoweth et al., 1993; O'Sullivan and Burrel, 2007), and anaerobic sediments containing decomposing kelp (Chynoweth, 1981) have not exhibited better decomposition rates compared to a sludge seed. Therefore, it seems reasonable that, for the case of anaerobic degradability testing where a standardized procedure is preferable, using seed sourced from a local wastewater treatment sludge digester would be suitable. Digested sewage sludge (DSS) contains a good broad spectrum inoculum, is abundant in supply and its successful use as seed has and is constantly being demonstrated (Owen et al., 1979; and ISO 11734, 1995).

Shelton and Tiedje (1984) found that in most cases, DSS diluted to 10% by volume is likely to contain all mineral and metal nutrients in ample supply, except for potassium (K^+), ammonium (NH_4^+) and cobalt (Co). For a DSS inoculum, sourcing from a first-stage or primary digester with a retention time of 15 to 30 days and organic matter (total solids x volatile solids) of 1-2% should be preferred. This is because a higher background gas production would result from use of inoculum with a shorter retention times or higher percent organic matter. Sourcing inoculum from a secondary or second-stage digester should be avoided as the digesters are usually unheated and unmixed with retention times of up to 90 days. Even though it has low background gas production, an attenuation of sludge activity may result (Shelton and Tiedje, 1984).

Sludge in the granular form (e.g. from an UASB reactor treating a brewery effluent) has been reported to exhibit a higher maximum methane production rate compared to its suspended form (e.g from a municipal sludge digester) (Neves et al., 2004). This suggests that the number of active bacteria involved in the acetogenic and methanogenic conversions was much higher in that structured sludge (granular form). The architecture and spatial arrangement of the granular sludge, protects the bacteria located in the inner core (mostly acetoclastic: refer Chapter 1) from adverse environmental conditions prevailing in the bulk medium. Neves et al. (2004) also found the use of granular sludge prevented acidification especially for waste/inoculum ratios between 0.5 to 2.3 g VS/g VS and alkalinity/COD ratio of 37 mg $NaHCO_3$/g COD.

Some have suggested that seed be grown in the laboratory and used as the master culture for batch tests. However, unless the batch tests that use the seed are done frequently and ongoing it may prove to be impractical to maintain a reactor requiring regular feeding and wasting. Heating and occasional effluent analyses are also required to monitor the status of the reactor. Vedrenne et al. (2008) for instance found that no significant effect of the origin of the inoculum was observed on the ultimate methane yield determination. Five different inocula were tested where three were of animal slurries (taken from farrowing sow, fattening pig and dairying cow) digested in the laboratory and two sourced from a winery and wastewater treatment plant digester, respectively. All the same, the time taken to reach 90% of the methane yield was significantly reduced with the use of seed from wastewater treatment plant (WWTP) digester or anaerobic digester of winery waste compared from a laboratory reactor. It took just 14 – 40 days with the use of the winery or WWTP digestate where an average time frame generally is between 62 and 72 days. The proportion of active biomass and the diversity of this biomass (hydrolytic biomass versus methanogenic) contained in each inoculum may have caused the observed differences.

2.4.7.2 Seed volume

A low seed volume is desired to minimise biogas production which may blur the contribution from the substrate. A low seed volume also increases the allowable size of the substrate sample. However, a restricted seed amount can cause process overloading due to the overproduction of volatile acids (Angelidaki and Sanders, 2004). For example, complete process failure was observed by Xu et al. (2002) when using a seed to substrate ratio (by volume, v/v) of only 0.5:1. No methane was generated and pH dropped from 6.7 at start to 4.0 at Day 2 which remained acidic (<5.0) thereafter. The lower the seed to substrate ratio, the greater the accumulation of the longer chain VFAs (Raposo et al., 2006). Where longer chain occurs acetic acid is likely to be present in the medium for extended periods, as it is continuously formed by the process of acidogenesis.

That said, Forster-Carneiro et al. (2008) saw an increase in methane production with the increase in inoculum content. The researchers concluded that the best performance for food waste biodegradation and methane production was when the seed to substrate ratio was 6.2:1 (v/v). A seed to substrate by volume ratio as high as 29.5: 1(2.3 L seed to 78 g substrate) resulted in a high maximum specific production per load (Raposo et. al., 2006). The maximum methane production per load can further be increased if more seed was added, as formation and stability of intermediate VFA products were already detected.

In addition to the volume of seed added, some researchers tend to dilute the seed for determination of ultimate methane yield. The idea behind this is to avoid a strong inhibition of methanogenesis by total volatile fatty acid (TVFA) (Vedrenne et al., 2008). This dilution could also be necessary to avoid inhibition of the hydrolysis by free NH_3. A dilution allowing a concentration of TVFA and free ammonia lower than 5 g L^{-1} and 100 mg N L^{-1}, respectively, is required to avoid inhibition of methanogenesis and/or hydrolysis. Vedrenne et al. (2008) when studying the effect of substrate dilution when testing methane potential of livestock slurry found that the CH_4 production was especially low for the undiluted incubation compared to a larger increase of methane production from diluted substrates. The time to reach the final ultimate methane yield also dropped from 101 to 73 days for undiluted to diluted incubation. With the diluted incubations, although different behaviours of CH_4

production were observed, all trials converged more or less to the same values of ultimate methane yield.

Diluting the seed leads unintentionally to a dilution of the reactor content which also means the total solids loading of the reactor is altered. The total solids contained in a reactor play an influential part in the anaerobic process performance. A simple one stage lab-scale, batch reactor requires feed material with total solids content below 10% whereas a more complicated and modern system can handle more than > 20% total solids content in the feed (Forster-Carneiro et al., 2008). Although the higher loading digesters allow for a more compact digester and high organic loading rate (OLR), the system is more susceptible to inhibition problems and harder to control. An increase in total solids loading in a batch reactor from 20% and 25% to 30% saw the gas production decreasing correspondingly. Total inhibition occurred at 30% total solids loading. This was probably an effect of overloading, with a methane production lower than the control. The contact of substrate and bacteria is likely to decrease at increasing total solids concentration. Instead, the specific methane yield at the end of the incubation period increased with a decrease in the source sorted OFMSW solids content (Angelidaki et al., 2006).

Clearly, for some substrates, increasing the seed to substrate ratio, with or without dilution, may be necessary for quick evaluation of total methane production. Mathematical means for estimating seed volume have been derived but they require an assumption of substrate hydrolysis constants (Angelidaki and Sanders, 2004), which are not easily estimated. The inoculums concentration affects the rate of biodegradation, the occurrence and duration of a lag period and the susceptibility of the degradation organisms to inhibitory effects (e.g. due to oxygen, toxic test materials, etc.). In addition, the background gas production increases in proportion with the amounts of solids present in the inoculum (Birch et al., 1989).

2.4.7.3 Seed pre-treatment

There may be opportunities to reduce the volume of sewage sludge used as seed by a simple, preliminary treatment. Birch et al. (1989) advised that for the reduction of the background gas production from inoculums the possibility of washing the sludge with dilution water (mineral salts medium) and pre-digesting the sludge in the laboratory prior to use can be examined. If the sludge from a sewage treatment plant is used directly, it is advisable to minimise any thermal shock to the micro-organisms during transport to the laboratory (Birch et al., 1989).

a) Phase separation

Separation is a way to produce manure fractions with higher gas potentials based on volume, since the water can be drained from the solids, giving these fractions a higher VS concentration (Moller et al., 2004). Digested sewage sludge (similar to undigested sewage sludge or mixed animal wastes) when left to settle typically separates out to three noticeable layers: a top floating scum layer, a watery middle layer followed by a bottom sludge layer. Kaparaju and Angelidaki (2008) optimized the phase separation when digested manure was settled for more than 24 hours and at a high temperature of 55°C. The total solids (TS) content of digested manure improved by 2.7 times for the top layer and 1.9 times for the bottom layer from the initial value of 3.9% TS. Ong et al. (2000a and b) found that the cumulative biogas production (400 L) of fresh cattle manure was highest for the top layer which also has the highest total solids of 12.7%. The middle layer with only 2.8% TS had the lowest cumulative biogas production (50 L). The methane potential difference between the various layers was attributed to the differences in the chemical composition of the particulate matter (Ong et al., 2000a).

There are several ways that the liquid or slurry can be separated. It has been reported that different separation techniques contribute differently to the ultimate methane yield (Moller et al., 2004). The ultimate and theoretical methane yield was determined in the solid fraction from a decanting centrifuge, from chemical precipitation, and from evaporation concentrate. A higher methane yield was observed from separation using evaporation (0.506 L CH_4/g VS), followed by centrifugation (0.247 L CH_4/g VS) and finally chemical precipitation (0.194 L CH_4/g VS). The time taken to achieve separation depends on the viscocity of the material and surrounding temperature (Kaparaju and Angelidaki, 2008). A higher rate and degree of separation was evident for digested manure undergoing separation at 55°C compared to 10°C. The time required for maximum solids separation for the former condition was 24 hours whereas the latter required a longer separation time of 120 hours. It seems higher temperature improves the separation process as the viscosity of the manure decreases with increasing temperature.

Most work regarding the phase separation involves some kind of manure and the methane potential that can be contributed by the various fractions. This means that during incubation, only the manure or its fractions are incubated and no other substrate was added. None has however realised the potential of the phase separation, and the effect of using the various fractions as a seed material for conducting anaerobic digestion tests. It would be valuable to see if the same findings here are applicable for digested sewage sludge (DSS), as DSS is one of the common seed materials. Earlier researchers have indicated the possibility for seed volume reduction best achieved using mechanical-thermal evaporation where water is evaporated from the liquid fraction pre-treated with a decanting centrifuge (Moller et al., 2004; Ong et al., 2000; and Kaparaju and Angelidaki, 2008).

b) Pre-digestion

Static pre-digestion of sewage sludge has been tested as a simple treatment. This treatment involves maintenance of anaerobic conditions, incubation at an elevated temperature, and typically without mixing. In the ISO 11734 (1995) and Pagga and Beimborn (1993), predigestion of the sludge was recommended when preparing seed for use in an anaerobic assay. Once collected from a local digester, the sludge inocula is allowed to digest in the laboratory at 35°C without the addition of any nutrients or substrates for up to seven days. Birch et al. (1989) predigested the DSS seed for 2 and 4 weeks, and found that by predigesting the seed, especially for 5 days, the background gas production was reduced. No unacceptable increase in either the lag or incubation period was observed from the predigestion. More recently, Hansen et al. (2004) also readapted seed from a thermophilic biogas plant to 55°C for three days before use in a batch anaerobic test. Moller et al. (2004) kept inoculum sourced from a farm-scale biogas plant in the laboratory at 35°C for 2 weeks before use in a batch test to remove most of the remaining methane production.

Factors such as (i) exposure to air, (ii) storage period, and (iii) storage temperature may influence the anaerobic metabolic activity of the stored DSS granules. Concerns have been raised regarding the maintenance of VFA (acetate, propionate, butyrate, etc.) degradation rate during long-term storage (Wu et al., 2008). The research by Wu et al. (1995) found that the length of storage period influenced metabolic activities of different microbial trophic groups. It took just 2 – 4 days to completely recover the degradation rates of acetate and propionate, following a storage period of up to two months. However, when stored for 18 months, the metabolic performance of the granules required 14 to 21 days to return to their original performance levels. This is because the acetate, propionate and butyrate degradation rates had dropped by 70, 50 and 80%, respectively. The syntrophic acetogenic bacteria (especially propionate- and butyrate-degrading bacteria) and acetate-utilizing methanogenic bacteria grow slowly since their doubling time ranges between 3 – 7 days under mesophilic temperature conditions.

The storage temperature had marked influence on decline of metabolic activities of different microbial trophic groups (Wu et al., 1995). At low temperature (4°C), degradation rates of acetate and propionate reduced gradually, but butyrate degraders sharply lost their activity by 45% in first month of storage. In contrast, degradation rates of VFAs declined gradually at 22°C. A direct correlation between temperature and decay coefficient was observed e.g. the higher the temperature, the more rapid was the decay coefficient. The VFA degradation and methane production performance of granules stored in a reactor at 22°C for 1 month can be recovered within 2 or 3 days, consistent with results from the batch test. Wu et al. (1995) concluded in his study that the important microbial trophic groups for AD can survive long term storage (up to 10 months) and their activities are recoverable. On the basis of costs and ease, storage of granules at laboratory temperature (about 22°C) and not at low temperature is recommended, especially for large scale application. Pre-digestion of the sludge allows re-adaptation of the collected inoculum to the intended test's temperature.

In addition, the level of anaerobically biodegradable substrate in the sludge is also reduced, removing micro-bubbles. A lower background gas production results which also allows for use of less dilute inoculums. However, some may argue that the added expenditure in time and effort are unnecessary. Further investigation of the effectiveness and suitability of predigesting the seed would be necessary before confirming its suitability over all substrates including solid organic samples.

2.4.8 Nutrient solution

The nutritional stimulation of methanogens has been reported by Speece (1988) where the microbial regeneration time is a function of the concentration of nutrients present. Nutrient limitations prolong the population doubling time of methanogens and at worst can cause generation eventually to cease altogether.

In a batch anaerobic test like the BMP test, the original nutrient media recipe as suggested by Owen et al. (1979) contained extensive listings of vitamins, micro- and macro-nutrients. The nutrient media are sometimes modified for example excluding vitamins when assaying maize in the experiment by Raposo et al. (2006). The nutrient media may typically contain ammonium chloride, potassium phosphite, magnesium sulphate, sodium phosphate, calcium chloride dihydrate, yeast extract, sodium carbonate, potassium dihydrogen phosphate, sodium bicarbonate, magnesium chloride, resazurin, ferrous chloride, and sodium chloride at varying concentrations (Raposo et al., 2006; Zhang et al., 2003; and Wu et al., 1995). The corresponding trace elements may include ferrous sulphate, manganese sulphate, cobalt chloride, calcium chloride hexahydrate, zinc sulphate, copper sulphate, boric acid, sodium molybdate, sodium chloride, sodium selenate and nickel chloride (Raposo et al., 2006; and Zhang et al., 2003).

Speece (1988) reported that the nutrients, in decreasing order of importance, are: nitrogen, sulfur, phosphorous, iron, cobalt, nickel, molybdenum, selenium, riboflavin and vitamin B_{12}. Ammonium appears to serve as the nitrogen source for all methanogens. Most methanogens utilize sulfide as a sulfur source, but some can utilize cysteine. Nickel is an essential component of bacterial ureases and in bacterial enzymes which convert H_2-CO_2 to acetate. It has been reported that 75% of methane is produced from acetate conversion (Evans, 2001). In addition to nickel, iron and cobalt must be supplemented to achieve high microorganism (volatile suspended solids, VSS) concentration.

Issues with nutrient supplementation generally are economic ones and toxic effects if excessive amounts are added. Economics will often dictate which nutrients can be justified for achievement of stimulated activity. Heavy metals are relatively inexpensive to supplement. Trace organics, like riboflavin and vitamin B_{12}, are costly which limits their commercial application.

Several researchers have looked into the significance of nutrient addition. A positive effect of nutrient addition was seen by Bogner (1990). When assaying solid landfill samples, methanogenesis was achieved more quickly when water and nutrient media were added to the BMP test. Average methane percentage of 30-50% was obtained within 50-60 days of batch incubation, compared to only 30% after 90 days without nutrient addition. Overall, the total gas production (in ml) tripled from un-amended controls and more uniform gas production were observed, reducing the variability in rates for the nutrient amended assays.

Nonetheless, some prefer to exclude nutrient or trace element additions in the batch test. Brummeler and Koster (1989) provided no additional nutrient in the batch test. Zhang et al. (2007) also avoided extra nutrients when analyzing food waste samples. The food waste samples collected from restaurants, food markets and commercial sources contained several significant nutrient elements such as carbon, nitrogen, phosphorous, potassium, calcium and magnesium. The macro and micronutrients were considered balanced for anaerobic microorganisms, hence the avoidance of extra nutrient. It seems that nutrient supplementation could be exempted if sufficient nutrients are suspected in the substrate. However, Bogner (1990) and (Gungor-Demirci and Demirer, 2004) seems to think that in order for nutrient to be exempted from a nutrient rich substrate digestion, sufficient amount of water need to be present to dissolve and distribute the nutrients that are included.

Another instance where nutrient supplementation can be avoided is when the seed is suspected to contain enough nutrients and that the seed volume is provided in an amount sufficient to prevent reactor acidification. Digested sewage sludge is likely to have all mineral and metal nutrients in ample supply, except for potassium, ammonium and cobalt (Shelton and Tiedje, 1984). Researchers like Birch et al. (1989) and Pagga and Beimborn (1993) has excluded the use of trace metals and sodium sulphide as they are suspected to be present in sufficient amounts in the inoculums especially in the presence of high concentrations of anaerobic sludge (3 g/l total solids).

It can be surmised that trace elements can be advantageous both if included or avoided in a batch anaerobic system. The need for supplementation seems to vary depending on the availability and accessibility of the nutrients in the test substrate and/or the seed. When extra nutrient is not necessarily required, no benefit to gas production can be seen. However, the nutrient supplementation proves to be fruitful under conditions where the nutrients and their accessibility are limited. A boost in gas production can be observed marking the importance of nutrient supplementation under this condition.

2.4.9 Sample pre-treatment

Several forms of sample pre-treatment have been reported for use in a batch methane potential test. Pre-treating the sample is important especially where mixed or heterogeneous waste is involved. The extent of sample pre-treatment also varies from a simple to a more extensive homogenization. A simple homogenization usually requires one pre-treatment step like blending the sample to a smaller and uniform particle size. Meanwhile, several more actions are taken under extensive homogenization. This may include first drying the sample, then crushing, grinding or milling it to a smaller particle size. Sometimes this step is repeated and water added to form smooth thick slurry.

Grinding and homogenising solid samples is thought to maximise gas production rates when measuring the BMP of landfill samples (Bogner, 1990). One study even pre-hydrolyzed its landfill sample for 2 hours at 1 atm in 0.1 N NaOH solution to transform solid waste samples to an easily degradable form. This is after the sample has been dried for 24 hours at 103°C and screened at 1.5 mm (Bilgili et al., 2009).

Extensive sample homogenization is also thought to influence the reproducibility of results (Hansen et al., 2004). Hansen et al. (2004) proposed a procedure for measuring methane potential of organic solid waste featuring extensive homogenization of the solid waste sample. The waste was first blended in a large industrial blender, then water added and sample further blended. Following that, the sample was transferred to a high-speed blender, diluted to a dry matter content of 10% and blended for about 5 minutes to achieve appearance of thick gravy. Gunaseelan (2004) for example, dried the fruit and vegetable solid waste samples at 60°C, then ground them in a blender to pass through a 2 mm mesh before analysis. Food waste sample has also been shredded, ground to 5 mm and mixed with tap water and inoculum to form a slurry like feed for methane batch test (Rao et al., 2000). When predicting anaerobic biogasification potential of ingestates and digestates of a full-scale biogas plant, the sample was first dried for 24 hours at 105°C and shredded in a blender to pass through a 2-mm mesh (Schievano et al., 2008).

Nonetheless, Chynoweth et al. (1993) thought otherwise. They concurred that no major changes to the extent and conversion rates of methane production when the biomass and waste feedstock were milled between 1-8 mm. Particle size reduction of samples such as meat, cabbage, potato and sludge cake to about 20 mm has been reported for a BMP test (Lay et al., 1997), but no studies with > 20 mm in 250 ml bottles. Other researchers too avoided extensive sample pre-treatment and instead opted for a simple homogenization. Heo et. al. (2004), Caffaz et al. (2007) and Schmit and Ellis (2001) crushed their food waste sample (sometimes down to 2-4 mm) using a cook mixer or blender but the sample was not dried prior. Charles et al. (2009) shredded the OFMSW to pass through a 25 mm screen with water added to provide sufficient moisture content of 55% which was part of the test objective.

Clearly there are many options to analysing the sample and the way the sample should be. However, with all the varied pre-treatments that have been performed, it is still unclear whether they are required in a batch test. This is especially when an extensive homogenization is involved when instead a simple blending or mixing may be sufficient to form a uniform feedstock. It would be informative to learn if extensive homogenization including sample drying and grinding to grain size (e.g. 2 mm) is really beneficial and required. Perhaps a better or similar reaction in sample degradation and methane production can be achieved without doing much damage to the sample prior to testing.

2.5 REPRODUCIBILITY AND REPEATABILITY

Reproducibility and repeatability are sometimes used as synonyms for precision. Repeatability might sometimes be called 'within-run precision', where analysts were to make replicate measurements in rapid succession, say an hour or so (Berthoux and Brown, 2002). The same set of reagent solutions and glassware were used throughout and temperature, feedstock, seed, humidity and other laboratory conditions would be nearly constant. Reproducibility might also be called 'between-run precision', where the same analyst was to conduct a number of measurements on different occasions. Difference in glassware, lab conditions, reagents etc., would be reflected in the result. It is generally expected that the between-run variability will be greater than within-run variability.

The performance of cellulose sample was used by Hansen et al. (2004) in a series of measurements for reproducibility and repeatability determinations. The work by Hansen et al. (2004) found that inhomogeneous inocula contributed to a high variation among three individual samples. In their opinion, cellulose samples having a difference more than 156 ml CH_4/g VS between the largest and smallest value in a set of three replicates is a sign that the result is unreliable and should be disregarded. The methane potential on average for the cellulose sample was 379 ml CH_4/g VS. If the average of the three control samples deviates significantly from a previously determined average, it may suggest that the inoculum, although evenly distributed in the reactors, was inhibited or for other reasons produced too little methane (Hansen et al., 2004).

A reduction in background gas production has been suggested to improve the precision of the test method (Birch et al., 1989; Vedrenne et al., 2008). The choice of seed for ultimate methane yield determination should be of seed that the final methane production should not exceed 20% of the total production (Vedrenne et al., 2008). If the production of the inoculum is higher, additional incubation with a lower biomass to substrate ratio must be performed.

Shelton and Tiedje (1984) checks the repeatability of the results by testing the standard deviation among within-run replicate bottles where reported values are of samples after removal of the seed blank. The repeatability is deemed to be good with standard deviation generally less than 10 percent, and could be expected to be < 15%. This finding was based on the results from digesting 94 organic compounds where 50 µg of carbon per ml was loaded into 160 ml bottles containing seed sludge. It was also noted that the standard deviation of the theoretical gas production of test substrate tended to decrease with increasing substrate concentrations. Pagga and Beinborn (1993) saw greater deviations of results when small digesters less than 150 ml volume was used.

That is why for biological experiments like a batch anaerobic digestion test, triplicate samples is important as a minimum (Hansen et al., 2004; Shelton and Tiedje, 1984). This is so that a sufficient number of samples for statistical analysis are available. It has been observed that the relative limited number of samples involved in the statistical analysis leads to a fairly coarse estimate of uncertainty and large confidence intervals. As more samples are determined these estimates most likely will improve. This could also partly be a function of sample size. For example, a number of 50 ml samples can be run, but one 2 L sample will be like running 40, 50 ml samples and finding the average performance because it aggregates effects. However, it doesn’t give an idea of variability like the 50 ml samples would.

It seems from the review here that some researchers suspected that the reproducibility and repeatability issues encountered during their anaerobic degradation tests is partly influenced by the quality of the inocula. The volume of gas coming from the inocula itself need to be low so that the result would be more precise. Although most researchers acknowledge the seed issue in test reproducibility, none has so far specifically addressed whether the seed indeed drives replicate variation and how. It may be that other factors could also be at play, for example the substrate condition, loading or test procedure and should be looked into to further confirm reproducibility factors.

PART 2: ANAEROBIC BIODEGRADATION KINETICS

2.6 INTRODUCTION

Anaerobic digestion is a multistep process, from the breakdown of complex compounds to the production of methane. The process has been described to proceed under three and sometimes four basic steps (where steps 3 and 4 as below are combined) (Veekan et al., 2000, Pavlosthathis and Giraldo-Gomez, 1991). First, in the hydrolysis stage, the complex organic materials, cellulose, lignins, proteins and lipids (fat and grease) are hydrolyzed by extracellular enzymes to soluble products. These are relatively simple, soluble compounds such as short chain fatty acids, and alcohols. This step can also produce carbon dioxide, hydrogen and ammonia. Secondly, in the acidogenesis stage, the products from the first stage are converted by microorganisms into acetic acid, propionic acid, hydrogen, carbon dioxide, and other low molecular weight organic acids. Thirdly, the acetogenesis stage sees the products from the acidogenesis stage being converted into acetic acid, hydrogen and carbon dioxide. Finally, methane is produced in the methogenesis stage through hydrogen and carbon dioxide reduction, and from acetate. About 70% of methane is produced from the latter, with the remaining from the former.

Kinetics provides the knowledge to predict the performance of digesters necessary for designing appropriate digesters. Kinetic studies are also helpful in understanding the inhibitory mechanisms of biodegradation (Rao and Singh, 2004). In general, the diverse literature can be sorted into three different categories, each describing a different approach to establishing the process kinetics. These are (i) the rate-limiting step approach, (ii) rates specific to each step of the anaerobic process, and (iii) the overall anaerobic digestion process.

2.6.1 The rate limiting step approach

Pavlosthathis and Giraldo-Gomez (1991) defined the ‘rate-limiting’ step as ‘that step which would cause process failure to occur in imposed conditions of kinetic stress’. In other words, for a multistep process like anaerobic digestion, the ‘rate-limiting’ step is identified as the step which proceeds at a significantly slower pace than the other reactions. This condition has also been called the ‘rate-controlling’ or ‘rate-determining’ step.

Lyberatos and Skiadas (1999) said the limiting step is not necessarily similar in all anaerobic digestion processes, especially if different operating conditions are imposed. Factors such as the hydraulic loading, temperature, wastewater characteristics among others may be the influencing force. Further information pertaining to factors influencing different limiting step in anaerobic digestion can be found under ‘Section 2.7.4 Hydrolysis rate constant.’

2.6.2 Rates specific to each major step of the AD process

The rates at which each step proceeds can be described as (Degirmentas and Deveci, 2004):

$$A \xrightarrow{k1} B \xrightarrow{k2} C \xrightarrow{k3} D$$

where A = the original complex organic material
B = hydrolysis products
C = volatile fatty acids
D = biogas
k1, k2 and k3 = reaction rate constants.

Examining the major steps separately, allows researchers to evaluate the specific reaction rate constants. Additionally, the condition that limits the anaerobic process can be discovered. In the study by Degirmentas and Deveci (2004), k1, the rate constant for the hydrolysis step, was smaller than the k2 and k3 values. Hence, it was concluded that hydrolysis is the rate-limiting step in the anaerobic treatment of antibiotic production wastewater.

A more complex model has also been applied where instead of the three basic digestion steps, the reactions are broken into even smaller steps. Viotti et al. (2004) simulates the anaerobic digester process through seven degradation subprocesses. This includes (in order) hydrolysis; amino acids fermentation; anaerobic oxidation of long chain fatty acids; anaerobic oxidation of middle-process products (propionate); transformation of acetate into methane and transformation of hydrogen into methane.

Siegrist et al. (2002) developed a model depicting the eight processes of degradation. The reactions are as follows, 1: biogas stripping, 2: hydrolysis, 3: fermentation of amino acids, 4: fermentation of sugars, 5: anaerobic oxidation of long chain fatty acids, 6: anaerobic oxidation of propionate, 7: acetotrophic methanogenesis, and 8: hydrogenotrophic methanogenesis. The behaviour of the anaerobic process at thermophilic and mesophilic conditions of anaerobic sewage sludge were successfully predicted using the Siegrist model. The biogas production, acetate and propionate concentration data showed that a mesophilic digester can withstand a substantial substrate loading without losing its operating stability provided no toxic compound is present. Although the thermophilic digester would allow 3-4 times shorter retention times, an HRT lesser than 8 – 10 days could result in a low hydrolysis rate.

2.6.3 Overall anaerobic digestion process

The most popular models for anaerobic systems are those that do not refer to the individual reactions discussed above, but rather assume methanogenesis as an overall process (Mata-Alvarez, 2003). This type of model usually considers the steady or stable state of the anaerobic digestion performance where the kinetic values are thought to represent both the acid-phase and methane-phase of the anaerobic digestion process. By uniting the many processes involved in the acid formation step (complex organic matter is hydrolyzed and acidified to produce VFAs by bacteria known as acidogens) and methane formation step (the product of acid phase are converted by methanogenic bacteria into methane and carbon dioxide), the anaerobic system model becomes more straight-forward and user friendly. It is well accepted that modelling anaerobic processes this way is not scientifically accurate and the ability to predict process performance is rather limited due to the lack of specificity in describing the phenomena involved in the overall process. The most common equation used to describe the overall reaction process is a first order kinetic model (Rao and Singh, 2004).

2.7 THE FIRST ORDER KINETICS

2.7.1 Introduction

First order kinetics has been commonly selected as the simplest model to illustrate the anaerobic digestion of complex substrates. The basic equation (Rao and Singh, 2004; Mata-Alvarez, 2003) is:

$$\frac{dS}{dt} = -kS$$

where k = first order kinetic rate constant ($time^{-1}$)
S = the biodegradable substrate concentration (mg/l)

The first order states that the degradation rate will be proportional to the degradable carbon concentration in the digester. The use of first order kinetics under continuous and batch systems are reviewed in the subsequent text.

2.7.2 At steady state (continuous system)

As written by Mata-Alvarez (2003), the first order application at steady state yields the value of S as a function of the hydraulic retention time (HRT):

$$S = S0 \frac{1}{1 + k.HRT}$$

where k = first order kinetic rate constant ($time^{-1}$)
S = the effluent biodegradable substrate concentration (mg/l)
HRT = hydraulic retention time (time)
S0 = the influent biodegradable substrate concentration (mg/l)

By substituting the S parameter with a B notation, the specific methane production as a function of the hydraulic retention time can be modelled instead. The mass balance is done by considering the concentration of biodegradable VS in the reactor is proportional to the gas production. This gives the equation:

$$B0.(S0 - S) = B.S0$$

where B = specific methane production in the particular reactor for each unit of volatile solids added,

B0 = ultimate methane yield in the same units

The two equations when combined, results in a simple model that can provide a single and useful kinetic constant (k). The larger k is, the faster the ultimate methane yield, B0 is achieved (Cecchi et al., 1991).

$$B = B0 \frac{K.HRT}{1 + K.HRT}$$

A high specific methane production can be realised through increasing the HRT. The highest specific methane production (B) that can be achieved would be the ultimate methane production of the substrate (refer Figure 2.6). This value is attainable at infinite HRT.

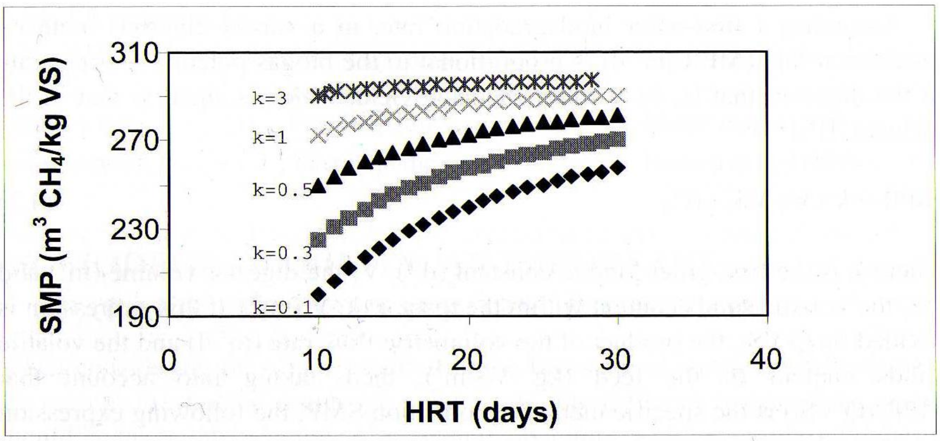


Figure 2.6 A simulated example for a substrate with B0 = 300 m^3 CH_4/kg VS. Kinetic constant k values are expressed in d^{-1} (source: Mata-Alvarez, 2003)

2.7.3 Batch system

Applying the first order basic equation to a batch system, this can be integrated into (Rao and Singh, 2004):

$$\frac{S}{S0} = \exp(-kt)$$

where	k	=	first order kinetic rate constant ($time^{-1}$)
	S	=	the biodegradable substrate concentration (mg/l)
	S0	=	initial substrate concentration
	t	=	incubation time (time)

Correlating the substrate concentration with biogas production (B), the following equation is obtained where B0 is the ultimate biogas production.

$$(B0 - B)/B0 = S/S0$$

By combining the above two equations, the anaerobic process for a batch system can be modelled as:

$$B = B0[1 - \exp(-kt)]$$

The model draws the relation between the volume of biogas production and digestion time through quantification of the first order biogas production rate constant, k ($time^{-1}$).

A typical graph of cumulative methane production over time in a batch system, when modelled using a first order, would see B zero at t=0 and the increase of gas production become zero at t=∞. In addition, the slope of the curves decreases as the incubation time progresses. This reduction in the slope with time can be attributed to the slowly diminishing concentration of the biodegradable substrate. Should a logistic S-curve be obtained, a stressed condition should be looked for including the presence of fast and slow digestible components and inhibitors (Chynoweth et al., 1993). One such event is if the seed to substrate ratio is low (Upadhyay et al., 2008). Imbalance was documented by Chynoweth et al. (1993) by the presence of higher concentrations of volatile organic acids in the assays with the lowest seed to substrate ratio. The first order rate constants for methane production were slightly higher for the higher seed to substrate ratios, indicating that increasing the seed to substrate ratio may be beneficial for some type of substrates.

The reduction of first-order rate constant at increasing substrate loadings was evident in the work by Sanchez et al. (1996). The sugar-mill-mud waste digested in a batch reactor saw the first order kinetic constant drop from 1.76 to 0.005 day^{-1} as the substrate loading rose from 15 to 27 g COD/l. The same effect of increasing substrate loading and decreasing first order rate constant was observed by Upadhyay et al. (2008), where k dropped from 0.79 to 0.11 d^{-1} with benzoate concentration increasing from 2500 mg/l to 8830 mg/l. Rao and Singh (2004) reported an increase in the methane production rate up to a substrate concentration of 59.74 g VS/l and decreased thereafter. The reactor fed with 135 g TS/l at 26°C, failed with VFA and pH at 19 g/l and pH 5.1, respectively. The accumulation of VFA at a higher substrate loading coupled with a lower inoculum volume was thought to have caused the reactor failure.

The first order kinetic model used in the study by Rao and Singh (2004) fitted the cumulative biogas production of OFMSW well and was able to portray the effect of different total solids loading, digestion time and temperature. Temperature also recorded a considerable effect on gas production especially at higher organic loading. This is confirmed by the higher biogas yield at 32°C compared to when incubated at 26°C. An increase in methane yield by up to 60% has been found when the temperature changed from mesophilic to thermophilic conditions (Converti et al., 1999). The effect was probably due to the more effective hydrolysis of polymeric materials at higher temperatures. On the contrary, Gunaseelan (2004) found that temperature had no effect on the methane yield of mango peels in his study on fruit and vegetable solid waste at 35°C and 28°C.

The first order rate kinetics has also been shown to be a good model at forecasting the biodegradability of the substrate. As in the study by Upadhyay et al. (2008), the biochemical methane potential and degradation rate constant were found to be less for phenol as compared to benzoate, signifying the higher biodegradability of the latter. In another study, when first order kinetics was applied on excavated landfill samples, it could help predict methane production amount at different landfill operational stages and the remaining organic fraction of the waste. This was done by varying the k and B0 value with respect to the operational time (Bilgili et al., 2009). The correlation between the model and experimental studies were determined to be more than 95% for all samples.

2.7.4 Hydrolysis rate constant

On top of predicting the overall performance of an anaerobic process, the first order model is also commonly applied to describe just the hydrolysis stage, where it is known as the hydrolysis rate constant. Neves et al. (2006) established the hydrolysis rate constant for coffee wastes were 0.035 – 0.063 d^{-1} with methane yields ranging from 0.24 – 0.28 L CH_4/g VS for varying compositions of coffee, barley, rye, malted barley and chicory in the waste. When these two values were correlated, an inverse linear relationship was obtained between the methane yield and hydrolysis rate constant. It is observed that when hydrolysis was faster, the methane yield was lower. First order kinetics helped establish that for coffee wastes, hydrolysis may not necessarily be the limiting factor to anaerobic treatment. Intermediates formed during the hydrolysis step may have been inhibitory to the methanogenic population. A material is deemed inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth (Chen et al., 2008).

Ammonia is a by-product of nitrogenous matter, like protein, phospholipids, lipids and nucleic acid decompositon and usually is found in two forms of inorganic ammonia nitrogen; the ammonium ion (NH_4^+) and free ammonia (FA) (NH_3) (Haug, 1993). At total ammonia-N concentrations below 600 mg/L, ammonia is beneficial to anaerobic processes since nitrogen is an essential nutrient for anaerobic microorganisms (Kayhanian and Tchobonoglous, 1993) but becomes inhibitory at concentrations between 1.7 to 14 g/L (Chen et al., 2008). Researchers like Sossa et al. (2004) and Kayhanian (1999) believe that the free ammonia form is the actual toxic agent reducing the activity of the methanogenic population citing 50 - 150 mg/L NH_3 as inhibitory concentration. The process becomes more inhibited at increasing pH due to the shift in NH_4^+ to the unionized NH_3 form from 1% NH_3 composition at pH 7 to 10% at pH 8 (Kayhanian, 1999). However, the opposite has been observed by Lay et al. (1997) for a well-acclimatized system where NH_4^+ was the more sensitive factor than the NH_3 level. The methanogenic activity dropped to zero around 6 g/L NH4+N.

The anaerobic process can be modelled based on the rate-limiting step which usually leads to a simple and readily usable model. Hydrolysis is normally considered as the rate-limiting step of the overall anaerobic digestion process if the substrate is in particulate/solid form (Lyberatos and Skiadas, 1999; Vavilin et al., 2001; Pavlosthathis and Giraldo-Gomez, 1991). However, it has been shown that the limiting step across different conditions and substrate is not always the same and could be influenced by pH (Zhang et al., 2005), long-chain fatty acids (LCFA) or ammonia (Lokshina et al., 2003).

After studying the degradation of cellulose, soluble starch and glucose, Noike et al. (1985) concluded that when refractory organic compound like cellulosic materials are digested, the hydrolysis rate of complex materials is the rate-limiting step. This follows the observation that hydrolysis of cellulose was so low compared to that of acetic acid, starch and glucose. If soluble hydrolyzed organic materials are fed into a digester, the methanogenic phase limits the overall digestion rate. The observations by Noike et al. (1985) are later repeated by Neves et al. (2008) when examining the biomethanation potential of restaurant waste. Assays with an excess of carbohydrates and protein presented higher hydrolysis rate constants (0.32 and 0.22 d^{-1}, respectively) than the wastes with an excess of lipids and cellulose (0.12 and 0.18 d^{-1}, respectively). Again, when lipids and cellulose are excessive, a slower hydrolysis is induced.

2.7.5 Overview of the IWA AD model

The Anaerobic Digestion Model No. 1 (ADM1) was developed by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Process for a generalised structure for anaerobic digestion modelling and simulation. The ADM1 is structured on the basic biochemical steps including disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis steps. The physico-chemical process is also included describing the ion association and dissociation, and gas liquid transfer (Batstone et al., 2002b). Process inhibition is also considered including pH, hydrogen and free ammonia (Batstone et al., 2002a). In this structured model, the first order kinetics is applied to explain the disintegration and hydrolysis process as well as biomass death.

The ADM1 model employs a large number of constants and coefficients. It describes 7 groups of bacteria and archea, catalyzing 19 biochemical kinetic processes, coupled to 3 gas – liquid mass transfer equations and 8 algebraic variables (Kleerebezam and van Loosdrecht, 2006). The complexity of the model is acknowledged by Parker (2005) when trying to calibrate the model against previously published reports on anaerobic digestion processes. Parker (2005) studied the application of the ADM1 model to several advanced anaerobic digestion such as a single-stage mesophilic digestion, acid phase digestion, temperature-phased anaerobic digestion and two-phase digestion and found a number of times, the recommended model parameters had to be used. The information required by the model is more detailed than is normally made available by the original researchers. Several other issues were also encountered affecting the applicability of the ADM1:

i) The model demands soluble and particulate COD values for the properties of the input stream into the digester. Where only volatile solids content is available, the accuracy of the prediction may be affected if an estimated COD value is used instead

ii) Where reactors operate at low solids retention times (SRTs), the model tends to overpredict VFA concentrations whereby improving for longer SRTs. At low SRTs the reactors tend to operate at reduced pH and the model predicted substantial inhibition of the acetoclastic methanogens due to low pH. It may be that the inhibition function for pH may over emphasize the impact of reduced pH on biological activity.

Concerned with the importance of detailed input required by the ADM1, Zaher et al. (2007) proposed a new procedure that accurately estimates the carbohydrate, protein, lipid and inert composition of complex solid wastes. The ADM1 considers the degradation pathways of carbohydrates, proteins and lipids, therefore inserting these information will enable a more accurate simulation of the solid waste anaerobic digestion process, The procedure developed by Zaher et al. (2009) estimated carbohydrate, proteins, lipids and inerts concentrations close to the values obtained from experimental analysis, with correlation coefficients (R^2) of 0.94, 0.88, 0.99 and 0.96, respectively. This was achieved by making use of the macronutrient elements of the substrates including carbon, hydrogen, nitrogen, oxygen and phosphorus and charge density, in addition to the practical parameters such as total solids, volatile solids, chemical oxygen demand, volatile fatty acid, total Kjeldahl nitrogen and total ammonia-nitrogen.

Four years since its initiation, Batstone et al. (2006) looked back at the implementation of the ADM1 in the research world. The review paper is a part of a special issue on the ADM1, and is especially published to establish current knowledge. The paper can be referred to for information of extensions, critical analysis and application of the ADM1 between years 2002-

2005. Discussions also investigate whether the ADM1 achieved its initial objective for a standardised anaerobic process modelling as well as assess any future requirements.

2.8 FIRST ORDER PARAMETERS

2.8.1 Method of estimation

Values of B0 and k that are sourced from the literature may not be suitable due to the variability in temperature control, apparatus, the substrate composition, seed and other factors. Therefore, researchers often turn to batch tests where the substrate is incubated at desired temperature until the gas production ceases. As shown by Chynoweth et al. (1993), using these experimental points, the B0 and k value can be estimated where the maximum methane yield at plateau and slope of graph, respectively (refer Figure 2.7). The first order parameters obtained from the graph serve as a guide. The parameters need to be estimated by linear or non-linear regression (Sanchez et al., 1996) or through the Thomas method (Lin et al., 1999).

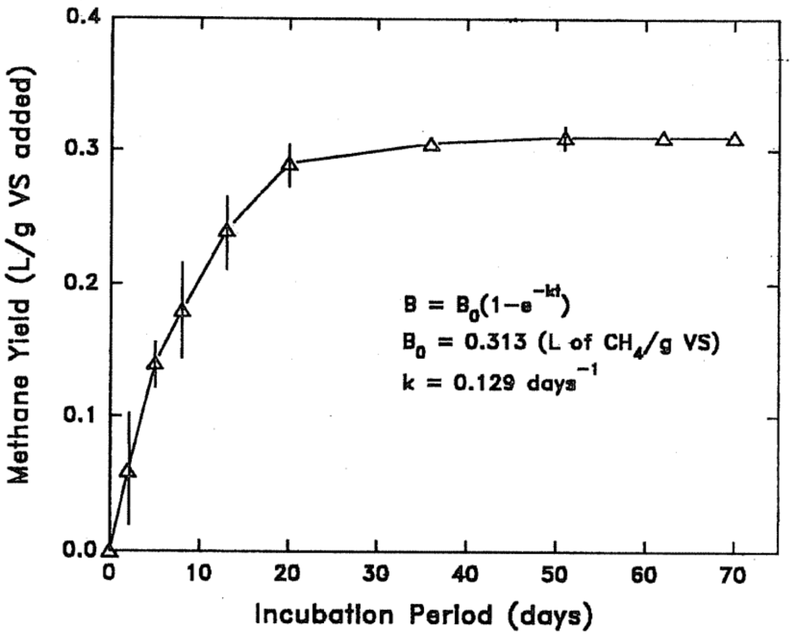


Figure 2.7 Typical biochemical methane potential curve of napiergrass (source: Chynoweth et al., 1993)

2.8.1.1 Linear regression

For estimating the first order parameters using a linear regression, the basic first order equation ($dX/dt = - K_h.X$) needs to integrated first into (Neves et al., 2008):

$$\ln\left(\frac{X}{X0}\right) = -Kh.t$$

where X is the substrate concentration (e.g. VS, COD) of the particulate substrate present in the assay at each time , X0 is the concentration of the particulate substrate initially present in the vial and K_h is the rate constant. The rate constant is established from the slope of linear plots of ln X versus time (refer Figure 2.8).

An example of fitting a first order model using linear regression can be found in the study of Gupta et al. (2009). The behaviour of batch anaerobic digestion of jatropha and pongamia oil cakes and their co-digested mixture with cattle dung was modelled using the first order kinetics.

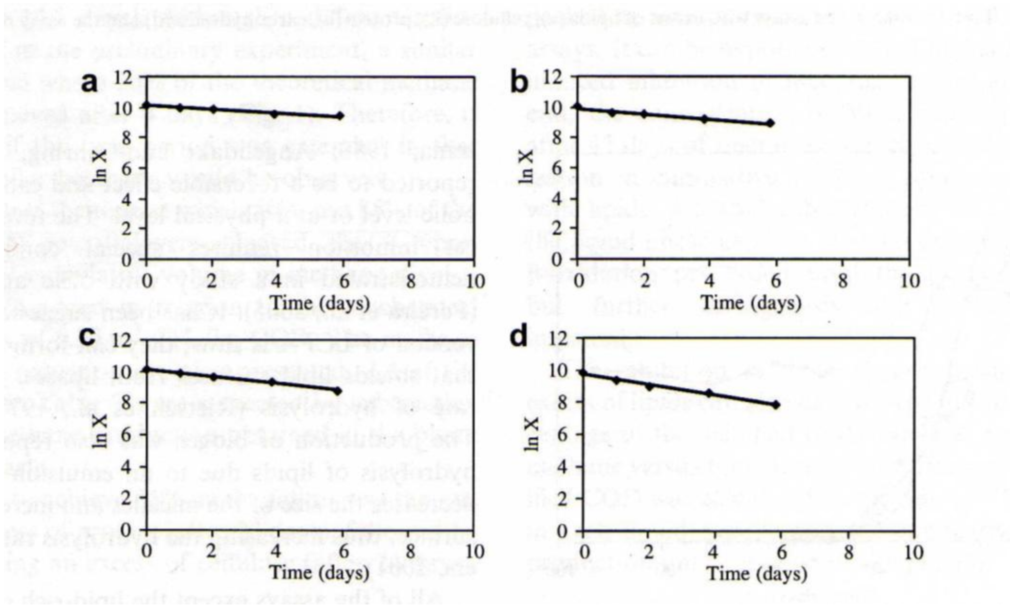


Figure 2.8 Linear plot of ln(X) versus time in the assays with an excess of (a) lipids, (b) cellulose, (c) protein and (d) carbohydrates. The rate constants were obtained from the slope of each line (source: Neves et al., 2008)

To ensure a best fit model, the adequacy and accuracy of the model fit are checked through error analysis. Error analysis is even more necessary if parameter estimation is done using linearization, whereby the nonlinear equations have been transformed to its linear forms. This in turn causes an alteration to their error structure, which may infringe the error variance and normality assumptions of standard least squares (Bhunia and Ghangrekar, 2008). Bhunia and Ghangrekar (2008) have looked at five different error functions to solve the non-linear regression analysis using the Monod, Grau second order and Haldene model. These are

The sum of the absolute errors (SAE)

$$\mathrm{SAE} = \sum_{i=1}^{n} |\mathrm{y\ calc} - \mathrm{y\ exp}|_{i}$$

The average relative error (ARE)

$$\mathrm{ARE} = \frac{100}{n} \sum_{i=1}^{n} \left| \frac{\mathrm{y\ exp} - \mathrm{y\ calc}}{\mathrm{y\ exp}} \right|_{i}$$

The hybrid fractional error function (HYBRID)

$$\mathrm{HYBRID} = \frac{100}{n-p} \sum_{i=1}^{n} \left[\frac{(\mathrm{y\ exp} - \mathrm{y\ calc})^{2}}{\mathrm{y\ exp}} \right]_{i}$$

Marquardt’s percent standard deviation (MPSD)

$$\mathrm{MPSD} = 100\sqrt{\frac{1}{n-p} \sum_{i=1}^{n} \left(\frac{\mathrm{y\ exp} - \mathrm{y\ calc}}{\mathrm{y\ exp}} \right)_{i}^{2}}$$

and

The sum of the squares of errors (SSE)

$$\mathrm{SSE} = \sum_{i=1}^{n} (\mathrm{y\ calc} - \mathrm{y\ exp})_{i}^{2}$$

The kinetic parameters of the various models are assessed by adjusting the parameters until a minimum value was obtained for each error function. In the case of Bhunia and Ghangrekar (2008), the error functions are minimized with the aid of solver add-in with the Microsoft's Excel spreadsheet.

The sum of the squares of errors (SSE) has also been called the error estimate (EE) by Masse and Droste (2000) with its percentage form termed as the percent of error estimate (PEE). After each simulation run, the percent error of estimate was calculated to evaluate the error between the measured and predicted values for each item.

$$\mathrm{PEE}\ (\%) = \left(\frac{\mathrm{EE}/\Sigma\left(\mathrm{Experimental\ value}_{\ i}\right)}{\mathrm{N}}\right) \mathrm{x}\ 100$$

2.8.1.2 Non-linear regression

A Quasi-Newton algorithm has been performed to estimate the values of model parameters by minimizing the sum of squared differences between the experimental and calculated values (Upadhyay et al., 2008). This method is also known as non-linear least squares fitting (Gavala and Lyberatos, 2001).

$$\mathrm{LSQR} = \sum_{i,t} \frac{\left(S_i^t - So_i^t\right)^2}{So\ max_i^2}$$

In general, the best method of estimating parameters will be the non-linear regression least squares. This is because the variables are used in their original forms and units. Errors are minimal when the kinetic constants are determined by non-linear regression methods (Bhunia and Grangrekar, 2008) When linearization is performed to transform the model to a form that can be fitted by linear regression, the estimates could be biased and imprecise. When possible, linearization should be avoided.

2.8.2 Displays of digester operation

Azeiteiro et al. (1998) offered an alternative to understanding a reactor operation by using a contour plot. Figure 2.9 shows the specific growth rate isolines are drawn as a function of an estimated parameter (acetic acid concentration) against the operating conditions (pH).

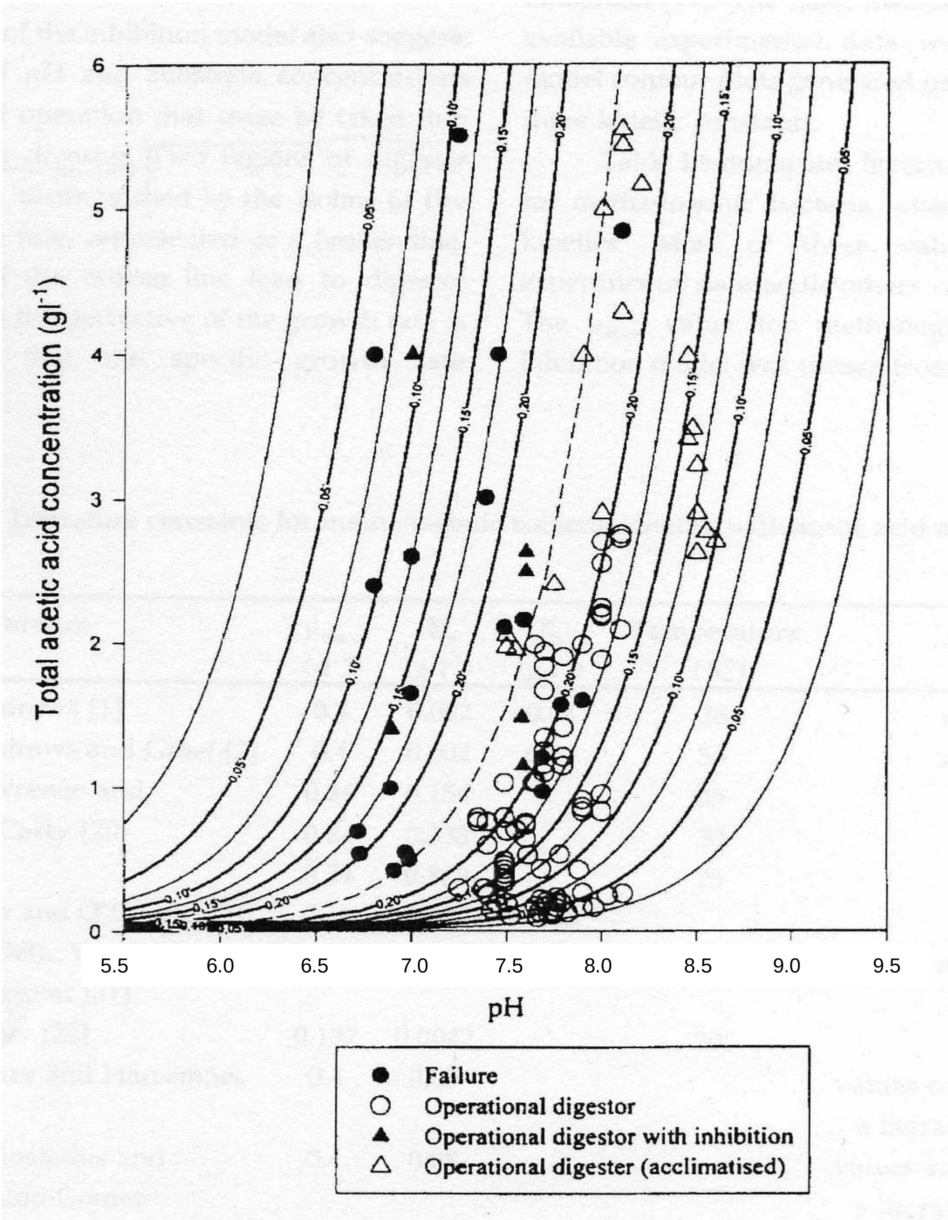


Figure 2.9 Contour plot showing effect of acetic acid concentration and pH on specific microbial growth rate. Broken line: represents maximum specific microbial growth rate (source: Azeiteiro et al., 1998)

The contour plot shows that for a digester to remain stable at pH 6.5, the total volatile acids need to be very low (below 500 mg/l). The minute distance between the isolines here implies that for the same pH values, similar concentrations of total acetic acid provide different microorganism growth rates. For pH values close to 7, it is possible to maintain digester stability with total acid concentrations of 1000 to 1500 mg/l. The inhibition function described by the contour plot warns that the inhibition is more severe at small pH values due to the high un-ionised concentration.

Two regions of digester operation can be defined from the isolines of the maximum specific growth rate, represented as a broken line. The values to the left of the broken line lead to digester failure following a decrease in specific growth rate as substrate concentration increases. Operation of the digester in this region must be avoided, by either increasing pH or decreasing the volatile acid concentration in the digester (decreasing the loading rate or diluting the digester content, for example). On the other hand, to the right of the broken line there is a stable region that can lead to successful operation of the digester. The application of the model may enable the behaviour of an anaerobic digester to be predicted from knowledge of total acetic acid concentration and pH.

2.9 WEAKNESS OF THE FIRST ORDER KINETIC MODEL

There have been several reported cases where first order kinetics was a poor fit and that other models such as Monod, Chen and Hashimoto, Step-Diffusional and Contois was more convincing. Cecchi saw that the biogas production rate of OFMSW over time in a pilot plant was not closely represented by the first order kinetics, signifying the lack of fit (Cecchi et al., 1990). The methane volume tends to be overestimated at the start and the end of the substrate incubation as well as underestimation of the methane production rate (Vavilin et al., 2004).

Because the first order basically is an empirical expression that reflects the cumulative effect of all microscopic processes, its tendency to not be able to simulate some processes is higher. Such was observed by Vavilin et al. (2004) when examining the hydrolysis stage of grey waste, they found Contois models allow better description of the initial phase of microbial colonisation of a surface of solid waste as well as the phase of a surface degradation.

An improper substrate loading could be a factor to as why first order may not work at times. The anaerobic biodegradation of medium strength anti-biotic production wastewater (10 $\leq$ COD $\leq$ 20 g/l) in the study by (Degirmentas and Deveci, 2004) was simulated by the first order kinetics reasonably well. However, when the wastewater concentration rose to COD $\geq$ 25 g/l, a second order kinetic provides a closer approximation (Degirmentas and Deveci, 2004). This was also the case for a batch system when for an initial substrate concentration greater than 29.5 COD g/l, the experimental data does not conform to the first-order kinetic model (Sanchez et al., 1996).

Converti et al. (1999) noted that substrate biodegradability could affect the applicability of the first order kinetics. Their work observed that the best prediction between experimental and theoretical values estimated by the first order model was of hemicellulose hydrolysate digestion (from 0.972 to 0.997). The worst was for the starch hydrolysate (from 0.957 to 0.966). The phenomena could be due to volatile acids accumulation causing wider deviations from the behaviour depicted by its experimental results (Converti et al., 1999). This is especially the case when digestion is performed on reasonably acidifying material (like starch) or where acidification is likely to occur (e.g. high loadings).

Another explanation could be due to the method used to estimate the first-order kinetic parameters. Prashanth et al. (2006) for example saw a disagreement between computed and experimental values when the Thomas method was applied to estimate the ultimate methane yield (B0) and first order coefficient (k). In fact, a number of B0 values deviated significantly from the experimental observations. It seems, the k value estimated with the Thomas method describes methane generation as the slowest step in the anaerobic process of soluble substrate. Prashanth et al. (2006) however found the experiments to be limited by substrate degradability and hydrolysis, and when an average k was used instead the predicted and experimental results correlated better. The average k was obtained by fixing B0 with methane at 60 days and varying the B with methane volume produced at day 5, 10 and 15. Their work indicates that a modification may sometimes be needed to fit the first order parameters, in this case an average k value is applied when substrate or hydrolysis limitation is suspected.

2.10 COUPLING BATCH AND CONTINUOUS DATA

It would be interesting to investigate relationships researchers have made between small scale laboratory tests to a larger scale pilot or even an industrial plant. The objective here is to recognize how the information learned in the laboratory was transferred to design of a large scale plant. This section aims to clarify this issue.

The anaerobic digestion of kitchen waste was studied in both batch and continuous modes by Mohan and Bindhu (2008). The batch study found the biodegradability of the kitchen waste was 83.5% and that a system loading of more than 4% TS could not be tolerated. The researchers then move on to digest the same material in a continuous reactor. It was not clear how the information from the batch test was useful; as the researchers went on to investigate a suitable OLR for the continuous system without any regard to the information gathered from the batch test. Anyhow, the optimum performance of the system was established at an OLR of 4.5 kg VS m^{-3} d^{-1} (15 days HRT) resulting in 81% and 79% of removal rates for chemical oxygen demand (COD) and VS, respectively. Maximum production of the methane was 0.288 m^3/kg of VS added. Using the published data of Mohan and Bindhu (2008), an inferred value of k was obtained as 0.07 d^{-1} and 0.04d^{-1} for the batch and continuous reactors, respectively.

Most researchers tend to compare rather than apply the information from the different reactor systems. For example, Kim et al. (2008) when studying the volumetric scale up from a bench scale process to a field pilot scale, found that the reduction rate of organic matter and methane production was comparable. The reduction rate of total chemical oxygen demand (tCOD) and the maximum methane content produced in the biogas from the bench-scale (0.40 m^3) system were 90.6% and 72%; whereas those from the field pilot-scale system (10 m^3) were 90.1% and 68%, respectively. The estimated methane yields were 282 and 254 l CH_4/kg tCOD $_{degraded}$ in bench and field pilot scale fermentation systems, respectively.

Similar comparisons were also made by Guendoz et al. (2007) where three different reactors were evaluated, a self designed lab-scale reactor (40 L), a pilot plant (21 m^3) and a BMP test (0.5 L). The reactors were fed with the same substrate. The researchers were satisfied with the lab-scale designed reactor as the methane yield was comparable to the pilot and lower than the BMP test. The lab-scale reactor has an average of 197 m^3 CH_4/tVS, while the pilot plant and BMP recorded 203 m^3 CH_4/tVS and 187 m^3 CH_4/tVS, respectively. Based on the published data, k is inferred as 0.09 d^{-1} for both the lab-scale and pilot-scale reactors. Yet, evaluating the new design based on the methane potential would probably be insufficient to confidently say that the reactor would behave similarly in an industrial scale.

Only one experiment can be found that correlates the kinetic constant, k, between different reactor operations (Chynoweth et al., 1993). In this work, a first order kinetic equation was used to model the overall behaviour of biomethanization of the OFMSW. The k value for the CSTR and BMP test was 0.035 d^{-1} and 0.046 d^{-1}, respectively for water hyacinth. Despite the value of k reported, the kinetics of the biodegradation was not discussed and detailed. It was not clear how the first order parameters were obtained, what estimation method was used, the precision of the estimation and so on.

2.11 SUMMARY OF LITERATURE REVIEW

There are many laboratory built and commercial tools that have been designed to measure the methane potential of solid substrates. The underlying concept is the batch incubation of substrate at specific temperature and an oxygen-free environment with the presence of seed to boost anaerobic decomposition. The variations to the test methods generally concern the addition or exclusion of nutrients, trace elements, buffer and sample pre-treatment. Some researchers opted to add these elements and conduct thorough sample homogenisation. Others prefer to test the substrate with minimal disturbance to the substrate and without other supplementations. Comparing the present state to way back in the 1970s, tremendous progress has been made in the determination of methane potentials. Of late, the tools have become more sophisticated with automation integrated into the device, allowing fast and easy gas and effluent analysis. Not only that, online monitoring and titration methods in the headspace allow for the supervision of reactors in real-time. Nonetheless with all the attention the methane potential tools have been getting, it does not mean to say that there is not more room for improvement. From the review of the methane potential determination the following points were observed lacking in integrity and requiring further investigation:

a) Reactor size

Most of the tools incorporate the use of glass bottles and rubber septum as the reactors. The glass bottles are typically 250 ml to about 1 L sizes. For liquid samples, this may not be a concern. However, problem may arise if a more heterogeneous sample for instance food waste is to be tested. The small glass bottles often see sample loading in the 50 g (wet weight) range which may be an ill representation of the larger sample. That is why for some conditions excessive sample homogenisation was performed such as sample blending, drying, grinding or hammer milled. An effect of these pre-treatment against testing sample as is has not been reported.

b) Mixing

There have been contradictory reports on the benefit of mixing regularly and less or no mixing. More reports on this matter by other researchers would further clarify this matter.

In Part 2 of this chapter, the first order kinetics application in predicting system behaviour under batch and continuous system has been reviewed. The first order is generally easy to apply but its consistency at predicting experimental behaviour varies with time. The first order seems to be less reliable when system instability or inhibition is suspected. Thereby, it is hoped that further use of first order kinetics in estimating substrate degradation and methane production by different researchers would help enhance understanding of its suitability. More study comparing the first order kinetics of a similar sample under different reactor system, batch and continuous is therefore warranted. The effect from using the k and B0 value from the batch test to a larger continuous system would help clarify reliability of using batch systems as preliminary tests for designing a large scale application.

Finally, complementary reports using different methods to evaluate reproducibility are required. Most researchers tend to evaluate reproducibility in terms of statistics, however, this is more appropriate when large data-sets are involved. Only one researcher has supplemented the estimation of kinetic constants by using contour plot (Azeiteiro et al., 1998). It would be interesting to see whether contour plot is also suitable to better understand the reproducibility of the estimated first order kinetic constants.

It has been the aim of this review to evaluate the shortcomings of current batch methods in determining the anaerobic decomposition of solid organic wastes. Additionally, reviewing the kinetics section helps point out the lack of information regarding the use of kinetics during reactor scale up. As outlined in Section 1.6, the objective of this research is to develop a new batch anaerobic respirometric device and apply it to estimate substrate biomethanization under a larger scale continuous system. The idea of this chapter is to gather points that need further refinements and compile knowledge which would be beneficial in achieving the objectives of this research.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

It is the aim of this chapter to illustrate the analytical procedures, sampling technique, and the instruments used in this thesis. It is easier to compile them into one chapter as their application is similar across all three results Chapters; Chapters, 4, 5 and 6. Nonetheless, the instance these procedures are applied plus its frequency varies and thus are detailed separately in each chapter.

A separate Methodology section appears in every result Chapter, expressing the specifics pertaining to their focus of study. This includes the specific experimental procedure, the test objectives, timeline, experimental set up and the number of replicates. The reactor operations such as the hydraulic retention time (HRT) or incubation period for the batch tests, substrate tested and its organic loading rate, mixing conditions etc. are also explained independently. As the data analysis differs for each result, they are therefore detailed explicitly in their respective chapters rather than in this Methodology Chapter. Chapter 4 which focuses on tube development mainly presents data in the form of yields and ultimate biodegradability. On the other hand, data are given in the form of kinetics for Chapter 6. Only issues regarding the reactor feedstock, the different reactor configurations, the analytical methods and suggestions for further improvements are discussed here in Chapter 3.

3.2 FEEDSTOCK

Several feedstock were used throughout this research specifically food waste, starch, cellulose, glucose, meat, sawdust, cabbage and rice. The corresponding solids and indication of the organic matter content is given in Table 3.1.

Table 3.1 The solids content of the feedstock

	MC% wet sample	TS% wet sample	VS%/TS%
Food waste	72 ± 6 (29)	28 ± 6 (29)	84 ± 3 (29)
Meat	65 ± 1 (3)	35 ± 0 (3)	86 ± 1 (3)
Meat & sawdust	84 ± 2 (3)	16 ± 1 (3)	80 ± 0 (3)
Cabbage	93 ± 0 (3)	7 ± 0 (3)	91 ± 1 (3)
Rice	71 ± 7 (14)	29 ± 7 (14)	99 ± 0 (14)
Glucose	0 ± 0 (3)	100 ± 0 (3)	100 ± 0 (3)
Starch	6 ± 1 (3)	94 ± 0 (3)	99 ± 0 (3)
Cellulose	6 ± 0 (3)	94 ± 0 (3)	98 ± 0 (3)

Note: MC: moisture content, TS: total solids, VS: volatile solids. Values after ± are standard deviations of the average, while values in brackets represent the number of samples

3.2.1 Food waste

For the most part, food waste as per Table 3.2 was used in experiments of Chapter 4, 5 and 6, consisting of 59% (by wet weight) vegetable, 29% leftover food, 8% teabags and coffee and 4% eggshells. The chemical and nutrient composition of the mixture is characterised in Table 3.3. The recipe for the food waste was adapted from a previous research for Master of Engineering thesis (Qamaruz-Zaman, 2005). However, this time, raw chicken bone and skin were excluded since these materials are almost impossible to be routinely homogenize using the domestic food processer.

In the work by Qamaruz-Zaman (2005), the waste mixture was developed after examination of numerous food waste collection trials (Moore et al., 2002; Farrell (2001); Steuteville and Karen (1996); Gies (1996), Viana and Schulz (2003); and Shin et al. (2000) highlighting the types of waste that are typically discarded and their relative portion in the overall collected

Table 3.2 The composition of simulated food waste by wet weight (g) and percentage (%)

Categories	Items	Wet weight (g)		%
Fruit and Vegetables	Potato skin	47		4.7
	Stir fried mixed vegetable	47		4.7
	Carrot skin	12		1.2
	Apple skin	22		2.2
	Apple seed	22		2.2
	Orange peel	77		7.7
	Broccoli stalk	72.5		7.25
	Pumpkin skin	42		4.2
	Pumpkin seed	27		2.7
	Salad	132		13.2
	Banana skin	92		9.2
			Σ	59
Leftover food	Rice	77		7.7
	Spaghetti leftover	57		5.7
	Mashed potato	57		5.7
	Bread	37		3.7
	Boiled pumpkin	61.5		6.15
			Σ	29
Teabags/coffee	Coffee ground & filter	45		4.5
	Teabags	33		3.3
			Σ	8
Eggshells		40		4.0
			Σ	4
	TOTAL	1000		100

Table 3.3 The chemical and nutrients characteristics of simulated food waste (wet weight)

Total nitrogen	0.41%
Total carbon	8.2 %
Phosphorus	0.039 %
Protein	17.9 % VS
Fat	12.6 % VS
Carbohydrate[1]	43.7 % VS
Lignin	8.4 % VS
Cellulose[2]	8.9 % VS
Hemi cellulose[3]	8.4 % VS

Note: [1]DM-ash-fat-protein-NDF (comprise of sugars and starches), [2]ADF-Lignin, [3]ADF-NDF (values of DM, ADF, lignin and NDF as in Table 4.6)

waste. These studies implied that vegetable wastes are predominant followed by grains and fruits with meat being the least discarded. Meats are usually bought ready trimmed and portion cut plus the leftover meat can be fed to pets.

The items for the food waste recipe were purchased from a local supermarket (Pak n Save) and prepared immediately at the researcher's kitchen. For cooked or hot items like rice, teabags, spaghetti, mashed potato, stir fried vegetable, filtered coffee and boiled pumpkin; they were left to cool to room temperature before weighing and mixing with the other ingredients. Approximately 20 kg of food waste were organized in one sitting which generally took up to three days. Once ready, the prepared food waste was transferred to the environmental laboratory in black rubbish bags.

At the laboratory, the food waste was blended to small pieces using a domestic food processor (Kenwood Multipro, China) without water addition (refer Figure 3.1 (a) and (b)). The main purpose of this sample pre-treatment was to obtain a reasonably harmonized sample which would otherwise be heterogeneous if left untreated, e.g., a large chunk of banana skin might be present in one reactor while absent in another. In order to eliminate this effect especially when conducting small scale batch tests, as well as to obtain a true representation of the whole sample, sample blending was thought necessary for this kind of substrate. The blended sample was then roughly divided into 1 L Glad Sandwich bags and stored in a freezer at -20°C (Fisher Paykel, New Zealand). When required for use, these samples were thawed overnight at 35°C.

(a) (b)

Figure 3.1 The condition of food waste (a) once prepared, and (b) after blending for use in experiments

3.2.2 Meat

Pet food (Buster Dog Roll) sourced from Pak n Save was used as a meat substitute as it solely contains minced meat and bones (refer Figure 3.2). It was opted over other meat sources like meat trimmings from butcher stalls or prepared meat waste, in order to standardise testing and to control for any content or size fluctuations when feeding the continuous reactors. Upon arrival at the laboratory, the pet food was stored immediately in the freezer at -20°C. When intended for use, the pet food was taken out from the freezer and adapted to 35°C overnight. The meat feedstock was prepared directly before feeding by cutting to smaller portions and manually mashing. This also sometimes included the addition of water and/or sawdust (Parkhouse Landscape and Garden Supplies, refer Figure 3.2). The idea behind sawdust addition was to slow the biodegradation process with the meat as a way to avoid reactor souring in addition to acting as a media to help retain microbes within the reactor. Water was added to help achieve a consistency with the feed that would be mixable using the manual mixing method as explained in Section 3.6.3.

(a) (b) (c)

Figure 3.2 The meat substitute (a) made from pet food (b) as shown when cut and (c) sawdust which was sometimes added

3.2.3 Cabbage

Cabbage was tested in the continuous reactors as it was assumed to be representative of a substrate high in cellulose. Cabbage bought from Pak n Save was cut to small sizes using a domestic food processor at the laboratory (refer Figure 3.3). The pre-treated sample was then stored in 1 L Glad Sandwich bags at -20°C while awaiting use, where defrosting overnight at 35°C was required.

(a) (b)

Figure 3.3 The (a) cabbage (b) the prepared cabbage as continuous reactor feedstock

3.2.4 Rice

Rice was the object of study in the batch tests, both tubes and bottles, as described in Chapters 4 and 6. The main idea was to check whether the reproducibility of test results was a function of the substrate heterogeneity and particle size, therefore rice was used as a standard substrate. Rice is reasonably homogenous in terms of its composition and particle size. Although other laboratory chemicals are available like starch or cellulose powder, rice was preferred as it is more representative of an organic solid waste. Sun Rice Basmathi rice brand (refer Figure 3.4) was purchased from Pak n Save and cooked at the researcher's kitchen the night before use. No sample pre-treatment was required and sample was tested as is in the laboratory.

Figure 3.4 The rice as feedstock in the batch reactors

3.2.5 Sucrose, starch and cellulose

Standard laboratory grade chemicals were used as sucrose (Chelsea White Sugar, New Zealand), starch (Analar BDH, England) and cellulose (MethoCel, Hydroxypropyl Methylcellulsoe, The Dow Chemical Co., USA) substitutes. These three substances were tested due to their different biodegradability; complex sugars (sucrose) and polysaccharides (starch and cellulose). Thus, their effect on potential methane production and allowable organic loading rate (OLR) can be identified through tests in the developed tubes (refer Chapter 4 for results). To control for any potential effect from sample heterogeneity and particle size, laboratory chemicals were used.

3.3 SEED

Except where noted, digested sewage sludge (DSS) was employed as seed for all the batch and continuous reactors. The exception was one occasion where laboratory grown seed was employed in the tube batch tests as it was the focus of study as discussed in Chapter 4.

The DSS was sourced from a mesophilic digester at the Christchurch Wastewater Treatment Plant (WWTP) in New Zealand with a 20 day residence time. The digester takes as input a mix of primary sludge and secondary activated sludge, both from a typical mix of residential, commercial, and small industrial sources. Seed was collected in 20 L plastic jars, filled to the maximum and capped loosely directly after filling. The distance from the WWTP to the laboratory is about 10.7 km with an average 26 minutes driving time. As the trip was reasonably short, detrimental effects due to temperature drop were not expected.

For most of the applications in this research, the seed was first acclimated to the laboratory conditions at 35°C for seven days before being introduced into the respective reactors. Typically, a 7 day old digested sewage sludge (DSS) had a pH of 7.2, 1% total solids (TS) with 69% being volatile (VS).

It is however acknowledged that DSS may be less suitable as seed in some circumstances. An example may be for the digestion of less degradable substrate such as woody materials (e.g. tree branches). In this circumstance, the use of other viable seed from other environments where anaerobic methanogenic decomposition of organic compounds occurs naturally may be more suited. Examples include anaerobic lake sediments containing decomposing kelps, the rumen of ruminant animals or animal faeces.

3.4 TRACE ELEMENTS

The trace element solution used throughout the research was based on practices at the laboratory of Waste Solutions, a company specialising in wastewater and environmental biotechnology located in Dunedin, New Zeland.The trace element recipe as listed in Table 3.4 was comparable to those used in other reported research (Owen et al, 1979; and Raposo et al, 2006). The solution had a pH of 4.0. The amounts of the solutions used in each reactor differs depending on the objective of study. The respective Chapters (Chapter 4, 5 and 6) need to be consulted for information on the trace elements usage in tubes, continuous reactors and bottles, respectively.

Table 3.4 Trace mineral solution (Chapman, 2006)

Item	Name	Weight (g) per 1000 ml
$FeSO_4.7H_20$	Ferrous sulphate	0.105
$MgSO_4.7H_20$	Magnesium sulphate	0.085
$MnSO_4.4H_2O$	Manganese sulphate	0.085
$CoCl_2.6H_2O$	Cobalt chloride	0.170
$CaCl_2$	Calcium chloride	0.100
$ZnSO_4.7H_20$	Zinc sulphate	0.210
$CuSO_4.5H_2O$	Copper sulphate	0.029
H_3BO_3	Boric Acid	0.010
$Na_2MoO_4 \cdot 2H_2O$	Sodium Molybdate	0.010
NaCl	Sodium chloride	1.000
$Na_2SeO_4.10H_2O$	Sodium selenate	0.017
$NiCl_2 \cdot 6H_2O$	Nickel chloride	0.024

3.5 ALKALI ADDITION

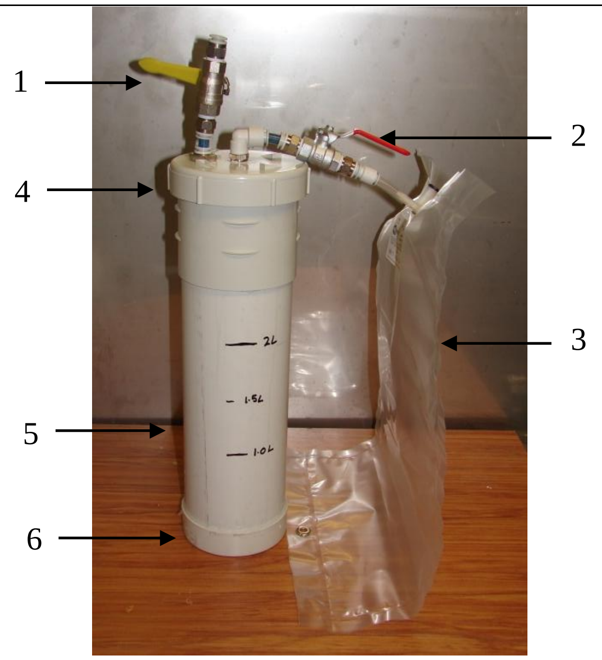
Alkali solution was made from a combination of sodium hydroxide, NaOH (Analar BDH, England) and potassium phosphate, K_2HPO_4 (Unilab, Alexandria). Each of the chemical at 50 g, were dissolved into 1 liter of deionised water. This addition of phosphorus (from the potassium phosphate) could also provide a nutrient benefit to the anaerobic digestion especially when incubating substrates with phosphorus deficiency. As shown in Table 3.3 there is only about 0.04% phosphorus in the food waste. The prepared alkali solution had a pH of 13.00 and was added to both continuous and batch reactors at specified volumes (refer Chapter 4, 5 and 6 for details) to raise the reactor pH.

3.6 REACTORS

Three different reactor configurations were used in this research. Chapter 4 focuses on the development of the tube batch reactors, Chapter 5 on the bench-scale continuous reactors while the bottle batch reactors are discussed in Chapter 4 and 6. For details on the organic loading rates (OLR) and hydraulic retention time (HRT) of the continuous reactors (CSTR) as well as incubation period for the batch system, the description can be found in the various chapters. All reactors are incubated at mesophilic temperature, 35 ($\pm$0.3) °C either in a walk in temperature controlled room or a freestanding incubator (Contherm Digital Series Cooled Incubator, New Zealand). Only the details pertaining to the design of the different reactors are described here.

3.6.1 Tube batch reactor

Figures 3.5 and 3.6 show the anaerobic respirometer designed and developed at the Environmental Engineering laboratory at the University of Canterbury, New Zealand. The tool, frequently dubbed as the 'tube', is made of 10 cm diameter polyvinyl chloride (PVC) pipe measuring 44 cm long with 3600 ml capacity. Caps are positioned at both ends, where one is fixed while the other is screw capped. All PVC materials were from Iplex Novadrain DWV (New Zealand) pipe and fitting range. The screw cap eases sample introduction while the valves are intended for biogas sampling and collection. The blue tubing which runs off the gas analyzer (GA 2000 Plus, Geotechnical Instruments, UK) allows reading of the tube headspace when it is connected to the valve of the tube. An AGA Gas Ball Valve – Magiflo (AAP Industries, Australia) was used as the valve and were connected to the lid of the tube using fittings by SMC Pneumatics (N.Z) Ltd. These fittings were a KQ2 Bulkhead Union, KQ2L Male Elbow and KQ2L Union Elbow, all with a 10 mm diameter. The protocol for using this tube is described in Chapter 4.



Description

1: Valve (remain closed until connected to a gas analyzer for gas analysis)

2: Valve (remain open when connected to a tedlar bag)

When gas analyzer is in use, Valve 1 will be connected to the inlet while valve 2 to the outlet.

3: Tedlar bag for gas collection

4: Screw cap

5: PVC pipe as reactor body

6: Glued endcap

Figure 3.5 The tube during incubation

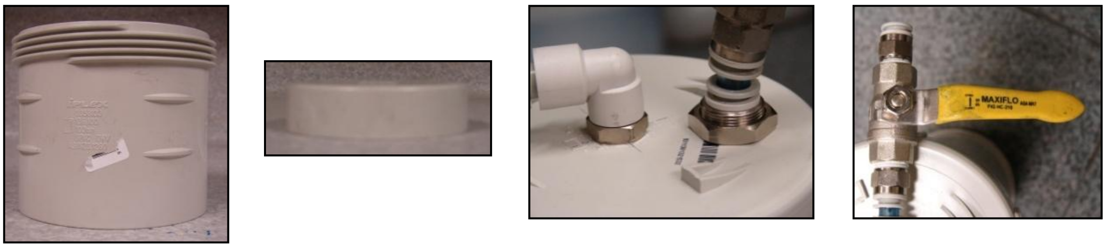


Figure 3.6 The parts and connections used in the tube

3.6.2 Bottle batch reactor

The 250 ml Schott Duran bottles are one of the commonly utilized vessels for conducting methane potential tests in the literature. The material made of glass along with the reactor capacity has been reported for biochemical methane potential (BMP) tests of solid organic waste as were other un-specified batch tests (refer Chapter 2). Thus, the bottle reactor as illustrated in Figure 3.7 was operated in order to imitate the widely accepted method for determining methane potential of a solid organic substrate using a batch system.

Figure 3.7 The 250 ml Schott bottles during incubation

The glass bottles were filled to a working volume of 200 ml leaving a headspace of 50 ml. Aluminium foil was wrapped around the bottle to provide a dark environment within the bottles. These reactors were mixed manually via wrist motion for about 20 seconds each day.

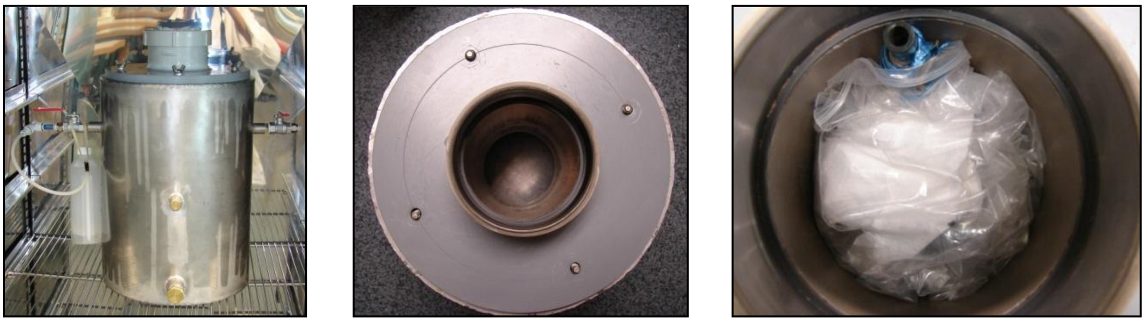
The seed and sample were first introduced in the bottles and mixed using a spatula. The lid coupled with a rubber septum is then fastened onto the bottle. After that, aluminium foil is wrapped around the reactor to provide a dark internal environment ideal for microbial growth. Using two syringe needles, for inlet and outlet, nitrogen gas is run through the bottle headspace for 20 seconds to set oxygen free conditions. These needles were detached and a 5 liter Tedlar Bag is then connected for biogas collection. This is done by linking a syringe needle fixed onto the bags into the bottle headspace. Mixing is performed daily using an orbital shaker.

3.6.3 Continuous reactor

A 30 L stainless steel cylindrical reactor built at the University of Canterbury Environmental Engineering laboratory was utilized as the continuous reactors (refer Figure 3.8). One of the concerns with operating the digesters for solid wastes were the feeding and wasting mechanism as well as the reactor mixing. Feedstock flow in and out the reactor through piping and the use of pump was ruled out. An internal stirrer controlled by motor was also deemed unsuitable for mixing. This was because the solid organic feedstock was quite thick and contains large solid particles. The condition of the wastes poses risks of pipe and pump clogging as well as mixing being inefficient or stirrer malfunctioning. As a result, alternative means was introduced.

A long and wide PVC pipe (the same as the batch tube) was fixed onto the reactor lid to function as the feeding and wasting chute (Figure 3.8). The pipe runs down straight into the waste's core inside the reactor, thereby preventing air to set in the digester's headspace, maintaining anaerobic condition (refer Figure 3.9). Still, a small amount of reactor contents are exposed to air during feeding or wasting, but are only limited to the surface layer which is inside the pipe in the digester (Figure 3.8). A bung made from stuffed paper towel filled into a plastic bag was inserted into the chute to minimise oxygen accumulation (Figure 3.8). When reactor effluent is to be exchanged, the bung was taken out. Wasting is done first by scooping effluent out with a long ladle through the pipe. Then, fresh feedstock is dumped via the same method.

A relatively easy, cost effective technique that offers considerable mixing was performed, simply by shaking the digester at an angle (refer Figure 3.10). To further ensure proper mixing, a baffled plate (Figure 3.11) is installed inside the reactor which picks up and dumps the waste as the reactor rotates. Mixing was done daily for 40 seconds before the reactor headspace gas is analysed and the reactor effluent exchanged.



(a) (b) (c)

Figure 3.8 (a) The continuous reactor during incubation, (b) the chute for inlet and outlet flow, and (c) the bung located inside the chute

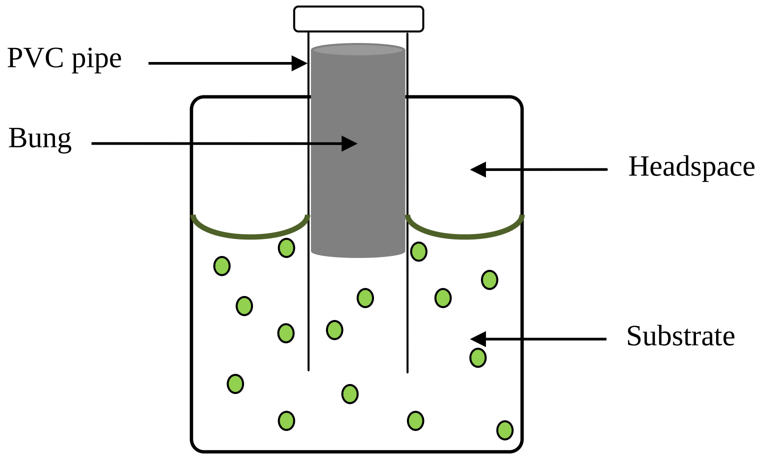


Figure 3.9 The position of the PVC pipe in the 20 L continuous reactor

Figure 3.10 The mixing mechanism by manually rotating the reactor on an inclined shaker

Figure 3.11 Baffled plate (marked by the oval shape) inside the continuous reactor for content mixing

3.7 ANALYTICAL METHODS

To get the overall picture regarding ongoing anaerobic treatment, the process performance and system stability needs to be evaluated. The process performance is checked through the influent and effluent solids content and the biogas production. System stability is indicated by the pH, volatile fatty acid (VFA), soluble COD (SCOD), ammonia (NH_3) and alkalinity of the reactor effluent taken progressively over the incubation period or at completion. Following are the descriptions of these analytical methods.

3.7.1 Gas analysis

3.7.1.1 Gas composition

Two methods were used to determine the biogas composition, namely via direct headspace analysis using a gas analyzer or through gas samples injected into a gas chromatograph. The former was suitable for vessels with valves attached to them; specifically the tube batch reactor and the continuous reactor. On the other hand, the latter was more appropriate for the smaller bottle batch reactors.

a) Gas analyzer

The gas analyzer as depicted in Figure 3.12 was a GA 2000 Plus (Geotechnical Instruments, UK) model capable of detecting methane (CH_4), carbon dioxide (CO_2), oxygen (O_2) and others (nitrogen, N_2, and other trace gas) (refer Table 3.5). The blue tubing (SMC Polyurethane 8x5 LRH.1, New Zealand) runs off the gas analyzer and is connected to the reactor to help regulate the headspace gas for analysis. A water and particulate trap is located at the inlet as shown in Figure 3.10, where a blue cone rubber housing holds a Sartorius 0.22 µm filter.

The gas analysis was performed for 60 seconds for each reactor. Methane and carbon dioxide reading is filtered to an infrared absorption frequency of 3.41 µm and 4.29 µm, respectively, while oxygen is measured by an internal electrochemical (galvanic) cell. Although other trace gases like hydrogen sulphide (H_2S), carbon monoxide (CO) and hydrogen (H_2) can be measured, special external pods and equipment reconfiguration are required. The equipment is sent to a certified calibration company, APC Services in Auckland for calibration every six months unless immediate attention is required. One or two occasions arose which necessitated such an action.

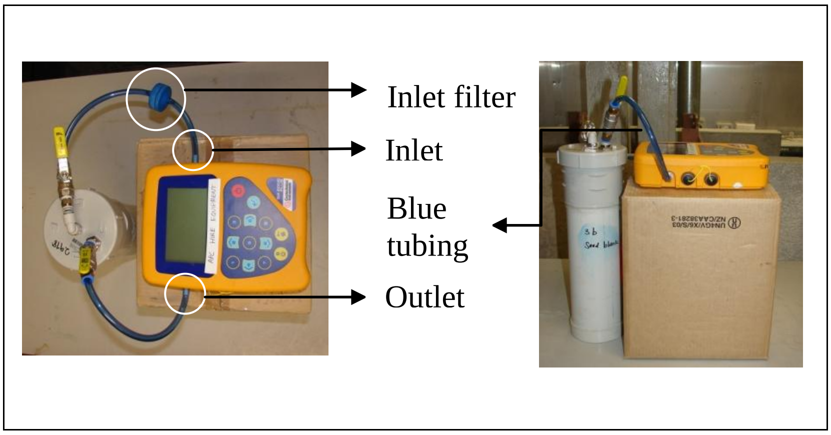


Figure 3.12 The gas analyzer when in use. Shown here is with the tubes. The same concept applies for the CSTR

Table 3.5 GA2000Plus specifications

Gas type	CH_4	CO_2	O_2
Range	0 – 100%	0 – 100%	0 – 25%
Gas accuracy	± 3%	± 3%	± 1%
Response time	≤ 20 seconds	≤ 20 seconds	≤ 20 seconds

When the GA 2000 is switched on, it will execute a 20 seconds self-check covering (i) general operation, (ii) pump function, (iii) gas flow measurement, (iv) calibration, (v) backlight function and (vi) solenoid function which then reports any upsets or malfunction. In one instance a ‘Low flow’ warning appeared while another, an ‘Over-pressurised’ warning. Checks at the calibration factory revealed that liquid or moisture ingresses had damaged the chemical cells with the first incidence, while the latter required new pressure sensors.

Repairing the instrument is not only costly in terms of money but also time. Therefore it is best to acknowledge the vulnerability of the instrument to excessive pressure and moisture/liquid. Based on experience, the following are advised when using the GA2000 gas analyzer:

i) An inlet filter is paramount to prevent particulates, dirt, dust and water from entering and damaging the internal sensors and cells. This filter needs to be used at all times and checked and replaced regularly.

ii) Try adapting the instrument at the test temperature for an hour before use to avoid condensation build-up in the tubing, filter or the instrument, especially if analysis is to done in a warmer environment.

iii) Because measurements are always taken after reactor mixing, the pressure build-up needs to be vented first. For example, in this case, release the accumulated pressure into the corresponding Tedlar bags before connecting the gas analyzer to the reactor.

iv) Always purge the equipment with ambient air for about 3 minutes say after 3-4 measurements before testing further, as well as before shutting down for storage.

b) Gas chromatography (GC)

For the batch bottle reactors, the biogas was sampled from the Tedlar bags equipped with rubber septums. A fixed 10 ml volume was taken each time using a gastight syringe (Hamilton Co., USA). The sample was then injected to a HP6890 Gas Chromatograph to analyse for methane, carbon dioxide and nitrogen content. Following are the characteristics of the GC gas composition method:

Column: J&W 19095P-Q04 HP-Plot/Q with 30 m x 535 um x 40 um

Gas: Helium with flowrate of 10 ml/min, pressure 11.13 psi

Oven: 50°C

Detector: TCD at 155°C

For each time the GC operates, a quality control check is carried out simultaneously. A standard gas mixture having 60.3% CH_4, 30.2% CO_2 and 9.0% N_2 and 0.5% H_2 is tested after four sample injections to ensure the quality of the chromatographs. As shown in Figure 3.13, the retention times for standard methane, carbon dioxide and nitrogen mixture (BOC Limited, New Zealand) is at 2.654, 3.314 and 2.543 minutes respectively.

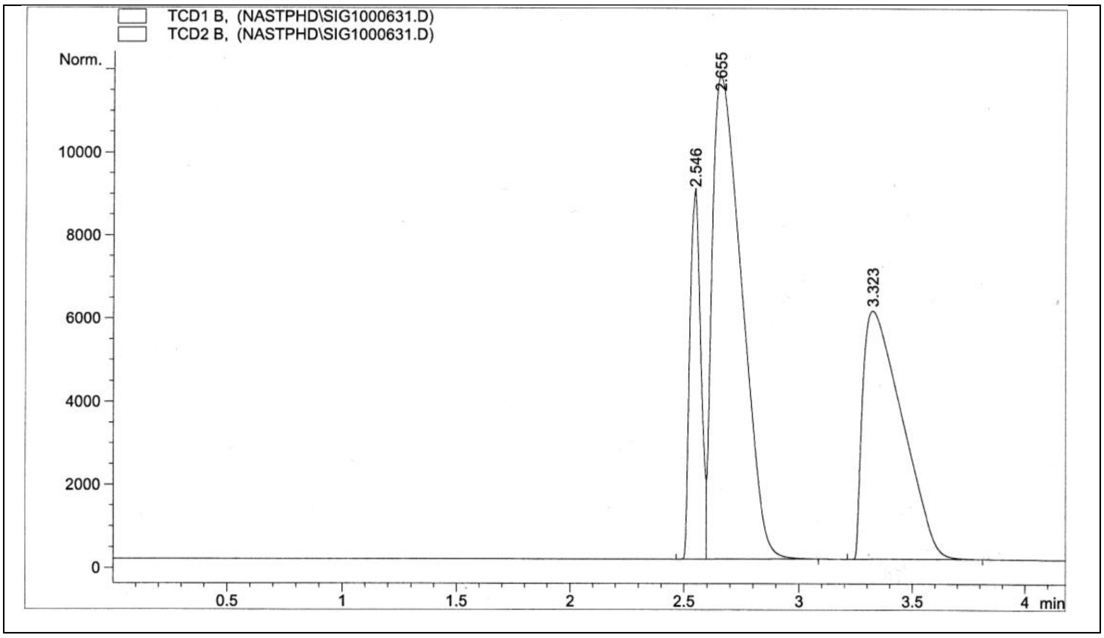


Figure 3.13 Retention time of gas standards

No significant problems were encountered with the instrument. The only shortcomings would probably be due to the sampling and analysis procedure which could be time consuming if a large number of samples are involved. To ensure sufficient gas sample is extracted by the syringe, the syringe plunger needs to be pulled slowly since the hole at the syringe needle is very fine. On top of that, the syringe needs to be allowed to equilibrate so that enough sample flows through into the syringe barrel. Consequently, when 20 samples excluding the quality control checks were analysed, gas sampling and analysis using the GC could take up to 3 hours. The inability to automate the GC instrument further adds to the impediment as the operator constantly needs to be present throughout the analytical procedure. To investigate the uncertainty of the gas composition analysis using the GC, the standard gas mixture was injected several times. The resulting analysis shows that an uncertainty of 3% can be expected with methane and nitrogen, while carbon dioxide has an uncertainty of 4%. A detection limit of 0.1%, 1.7% and 0.8% is estimated for the methane, carbon dioxide and nitrogen gas respectively.

3.7.1.2 Gas volume

For all reactors the concept of determining gas volume is by gas collection in an external container. This literally means collection in Tedlar bags for the batch tubes and bottles, while balloons made to retain helium were used for the continuous reactors, as illustrated by Figure 3.14. The Tedlar bags were from SKC Inc (USA) and were of 1 L and 5 L sizes equipped with single polypropylene septum fitting which combines the hose/valve and the septum holder into one compact fitting. The smaller bags were more suited for the bottles and the tubes with seed blanks. The helium balloons (18 inch diameter foil balloon, Party Warehouse, New Zealand) were employed as the material accommodates well to high gas productions compared to a 10 L Tedlar bag. The balloons generally can collect to a maximum of 13 L biogas. More importantly, the helium balloons were more robust for long term use compared to the Tedlar bags. Often, when 10 L Tedlar bags were initially tested, the bags leaked especially near to the septum and connections.

A typical oxygen content in the reactors rarely exceeds 0.5%, and often this oxygen level drops to 0.0% after sometime (< 60 seconds). It seems probable the oxygen is contributed from the plastic tubing of the gas analyzer used to measure the reactor headspace. However, the oxygen contributed by this instrument is at very low levels and gets consumed by microorganisms within the reactor quickly. A damaged Tedlar bag or balloon is ruled out as a a possible factor for reactor oxygen contamination. This is because it requires a sudden drop in the gas volume to suspect a bag or balloon malfunction. This also means the possibility of oxygen diffusion from the atmosphere into the gas containers is very unlikely. To check if leaking has occurred, a filled bag or balloon is immersed in water and the formation of bubbles are looked for.

To ascertain the volume of gas collected, the containers are connected to a water displacement device (gasometer) as depicted in Figure 3.15. The smaller scale gasometer 500 ($\pm$ 5) ml is more precise for low gas productions e.g. from the gas bottles or after 14 days incubation for the tube reactors, while the 5 ($\pm$ 0.247) L gasometer was ideal for the continuous reactors. The former has an accuracy of $\pm$ 1%, while the latter $\pm$ 5%. Pressure is applied manually onto the bags or balloons by pressing them gently by hand. The biogas then flows into the gasometer displacing the liquid (tap water), and in turn raising the inverted cap. For the 500 ml gasometer, the volume is read off directly by levelling the internal and external meniscus. The larger gasometer had a weight and ruler at the side where the corresponding level in cm is read off. Then, the value is times by 246.6 to obtain the volume as 1 cm equals to 246.6 ml.

(a) (b)

Figure 3.14 The gas collection means (a) Tedlar bags (1 L and 5 L) for the batch systems and (b) helium balloons (13 L) for the continuous reactor

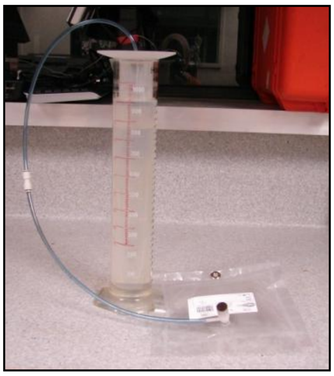
It is well known that methane is hardly soluble in water whereas a considerable fraction of carbon dioxide can dissolve. A highly acidic or saline solution has been reported to prevent the dissolution of carbon dioxide in the liquid phase (Rozzi and Remigi, 2004). Using sulphuric acid (H_2SO_4), salt solution (NaCl) and tap water (H_2O), an investigation on the effect of different barrier solutions in the water displacement device was performed. Table 3.6 shows the three solutions perform almost equally. Having acidic solution in the water displacement did not prevent CO_2 dissolution better than a tap water would, resulting in 17.4% and 17.3% CO_2 loss, respectively. The most inefficient barrier solution type would be saline as the CO_2 loss was higher, at 19.0%. It is believed that the type of liquid used does not impact much on carbon dioxide gas rates. Further dissolution of carbon dioxide is possibly avoided once equilibrium between the liquid and gaseous phase is reached (Rozzi and Remigi, 2004). Considering the findings here, it is decided that tap water would be use in the water displacement device in this research. It is anticipated that the results reported in this thesis will not be impacted by the 17% loss in CO_2 for two reasons (i) the CO_2 composition in percentage is read off directly from the reactor headspace using a Gas Analyzer (ii) and CO_2

dissolution is minimized because pressure build-up is avoided (the biogas produced moves to an external Tedlar bag).

Table 3.6 The carbon dioxide loss depending on the barrier solution

Barrier solution	CO_2 % after passing through barrier solution	Average CO_2 % loss*
Acidic	31.4	17.4
H_2SO_4, 5%	29.6	
	25.0	
Saline	26.5	19.0
NaCl 200 g/l and	30.1	
citric acid 5 g/l	27.7	
Water	30.0	17.3
Tap water	30.6	
	25.5	

*The starting carbon dioxide percentage is 34.7



(a)

(b)

Figure 3.15 The (a) 500 ml and (b) 5 L water displacement devices

3.7.2 Influent/Effluent analysis

The reactor feedstock and digestate contained large particles and was of thick slurry which poses problems for sample extraction. Because analysis on fresh sample was difficult, filtered and diluted samples were opted for instead. Sample pre-treatment involves centrifugation at 4.3 rpm for 15 minutes to separate out the supernatant. Centrifugation was effective at separating out roughly 80% of supernatant from the total volume. Having a more refined liquid was helpful for the filtration stage as the filtration time and events of clogging is reduced significantly. The supernatant was filtered with a glass microfibre filter (Whatman, grade GF/C, 1.2 μm pore size) to obtain a clearer filtrate. This filtrate is diluted ten times with deionised water, ready for SCOD, ammonia, alkalinity and VFA testing. The pH and solids content is determined on raw, fresh samples.

3.7.2.1 pH

The pH value was taken with an EDT RE357 Microprocessor pH meter (Made in England) and reported to one decimal place. The performance of the pH meter is checked daily using standard solutions at pH 4, 7 and 10 (Scharlau Chemie S.A., EU). Instrument will be calibrated at the laboratory according to the Instruction Manual Series 3 if deteriorating quality is detected.

3.7.2.2 SCOD –Hach Method 8000

Because COD was analysed on filtered sample, the value is of the soluble form and not total COD, thus termed SCOD. SCOD analysis is performed using the Hach Dichromate Reactor Digestion Method test, where small volumes of sample are pipetted into vials containing the potassium dichromate in a 50% sulphuric acid solution as reagents. The COD values are determined colometrically on a Hach Spectrophotometer using program #435 where the detectable COD concentration ranges from 0 - 1500 mg/l. The method involves sample digestion for 2 hours at 150°C, and then examining the digested sample at a 620 nm wavelength. Samples are diluted with distilled water if the SCOD concentration falls over range and the same test procedure is repeated. The uncertainty of the SCOD test is estimated to be 5%, obtained by analysing a sample three times. The triplicate analysis gave a sample SCOD concentration of 28000 ($\pm$ 1290) mg/l at 25 times sample dilution.

3.7.2.3 Ammonia (NH_3-N) - Hach Method 10031

Ammonia was determined using Hach Test 'N Tube kit consisting of High Range Amver Diluent, Ammonia Salicylate and Ammonia Cyanurate as the reagents. The method was Method 10031, the Salicylate Method. After a 20 minutes reaction time, a green colour will develop if ammonia is present in the sample. Using the Hach Spectrophotometer under program #343, the ammonia concentration between 0 – 50 mg/l can be detected at a wavelength of 655 nm. If the ammonia value is off-scale, samples will be diluted with distilled water and reanalysed. A sample was analysed three times in which the uncertainty of the ammonia test was found to be 4%. The ammonia concentration of the sample was 1225 ($\pm$ 50) mg/l where a 25 time dilution is needed for the sample to fall within range.

3.7.2.4 Alkalinity – 2320 B

Alkalinity is done via manual titration as guided by the Standard Methods (1989). Sample is titrated with 0.2 N H_2SO_4 to a pH of 4.5. The concentration is calculated using the formula:

$$Alkalinity\ (mg\ CaCO3/l) = \frac{A\ x\ N\ x\ 50000}{sample}$$

Where A = volume acid used (ml)

N = acid strength (mg/l)

Sample = 100 ml

3.7.2.5 Volatile fatty acids

For the volatile fatty acid (VFA) determination on the HP6980 Gas Chromatograph, further sample filtration to 0.22μm is necessary to prevent GC malfunction. The method for VFA analysis is as follows:

Column: HP 19091N-133, HP INNOWax Polyethlene Glycol with 30m x 250μm x 0.25 μm

Gas: Nitrogen with flowrate of 2.2 ml/min, pressure 27.06 psi

Oven: 120ºC for 1 min increase at 10ºC/min to 250ºC hold at 250ºC for 2 min, decrease temperature to 120ºC hold at 120ºC for 0.5 min

Detector: FID at 300ºC

The volatile fatty (acetic, propionic, butyric and valeric acids) were identified by comparing the retention time with that of known standard. Only these VFAs were examined as many researchers (Wang et al., 2002; Lokshina et al., 2003; Bouallagui et al., 2004) have reported these acids as the significant fractions of VFAs in organic wastes which are usually reported as lumped isomers.

Based on the chromatogram in Figure 3.16, the retention time was 2.489, 3.037, 3.717 and 4.062 minutes for acetic, propionic, butyric and valeric acids, respectively. Conversely, all four acids were summed together to give a total VFA (TVFA). The concentration of acids is expressed in mg/l by comparing the total peak areas of the chromatogram to the total peak area of the external standards. This is usually done by plotting a graph of area versus concentration, known as a calibration curve (refer Figure 3.17). An uncertainty of 2.3%, 1.3%, 2.2% and 2.6% is estimated for the acetic, propionic, butyric and valeric acids, respectively. Correspondingly the method detection limit for these acids are 313 mg/l for acetic, 188 mg/l for propionic, and 172 mg/l for butyric and valeric acids.

To ensure the reliability of the GC results, a pure acetic, propionic, butyric and valeric acid standard was always tested after three sample injections. On top of that, all standard and sample injections are done 3 times and the average value is reported. If a deviation from the standard concentration was observed, the instrument was checked. This was first done by confirming the earlier observation by further conducting analysis on individual VFAs whereby various concentrations were tested.

During the course of this research, there was only 3 times where instrument deterioration was suspected. For each of these occasions, the situation was rectified by cutting about 10 inch off the column. This was because column degradation was suspected which was probably an effect from sample overloading, poor sample focusing on the column or contaminants trapped at the head of the column. Cutting some of the column especially the degraded part helps improve the retention time reproducibility, the peak area precision and repeatability of the chromatogram. This indicates that the samples tested here were thick, sticky or dirty, and needs to be scrubbed to obtain a cleaner sample. Unfortunately, a rigorous sample pre-treatment such as solvent extraction etc. may result in a sample less representative of the original.

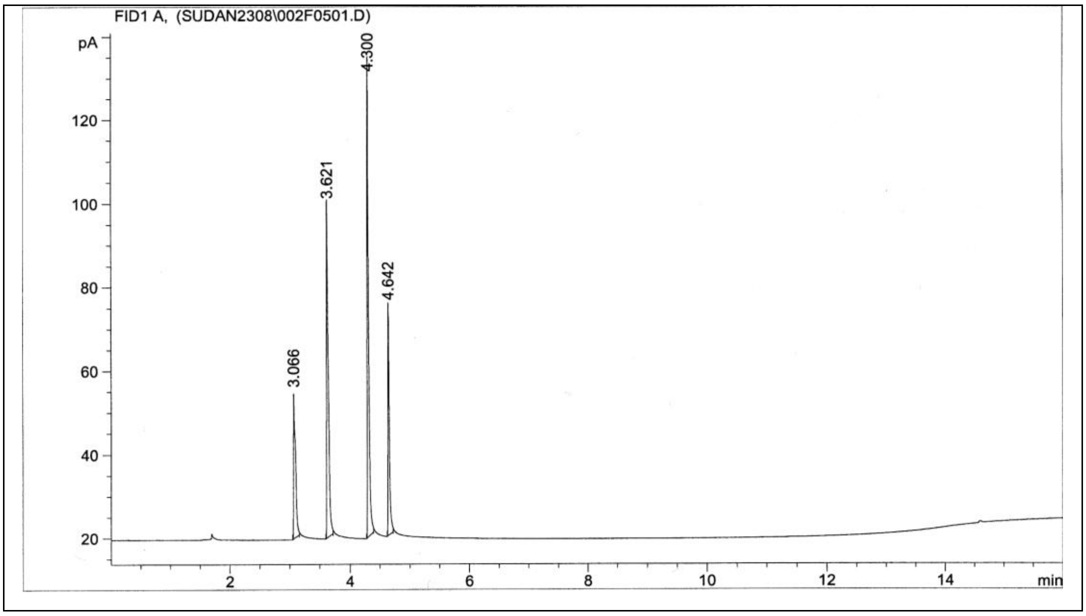


Figure 3.16 Retention time of VFA standards

Once the problem with the GC was resolved; the instrument was again checked by testing varying acetic, propionic, butyric and valeric concentrations. In all three cases, a satisfactory response was achieved and analysis on test samples was resumed.

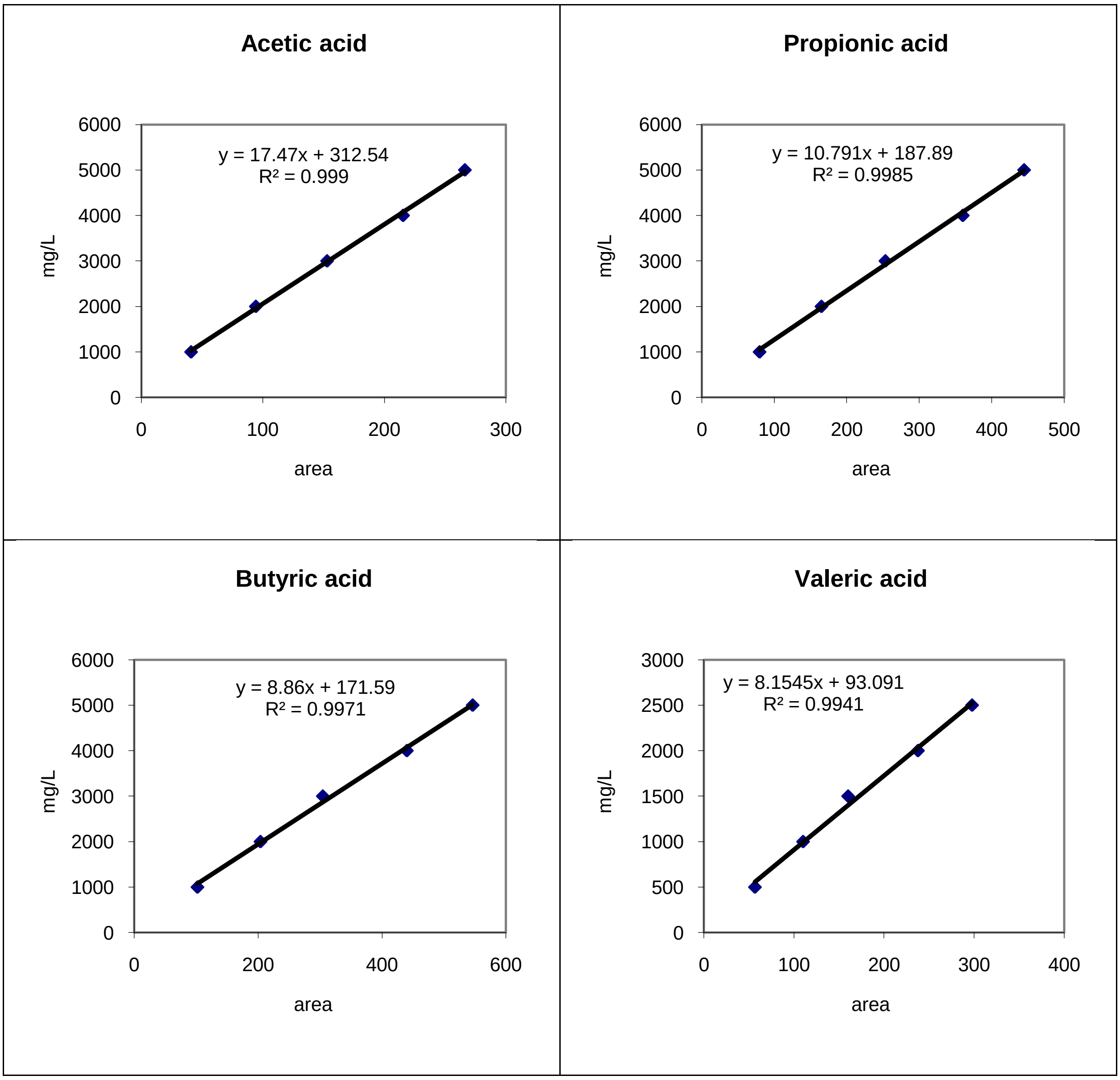


Figure 3.17 The calibration curves for the individual VFAs

3.7.2.6 Solids content

The influent and effluent was tested for the total solids, volatile solids and moisture content based on the Standard Methods (1998). Each of these is described below:

a) Total solid, TS (Standard Methods 2540G)

A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the total solids. The total solids in percentage of wet sample are calculated as:

$$\% \, Total \, solids = \frac{(A - B) * 100}{C - B}$$

Where A = weight of dried residue + dish (g)

B = weight of dish (g)

C = weight of wet sample + dish (g)

D = weight of residue + dish after ignition (g)

b) Total volatile solids, TVS (Standard Methods 2540G)

The residue from the total solids determination is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. Total volatile solids are determined as per calculation below. Its determination is useful in the control of biological treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastes.

$$\% \, Total \, volatile \, solids = \frac{(A - D) * 100}{A - B}$$

Where D = weight of residue + dish after ignition (g)

3.7.2.7 Moisture content (Vesilind et al., 2002)

The moisture content is the lost of weight after drying a sample to a constant value in an oven at 103°C to 105°C. The expression for calculating moisture content as per below is on wet basis.

$$\% \, Moisture \, content = \frac{(w - d)}{w} * \, 100$$

Where w = initial (wet) weight of sample d = final (dry) weight of sample

3.8 SUGGESTIONS FOR IMPROVEMENT

The methods used for this research were adequate for the purpose of study as set out in the Introduction Chapter. Nonetheless, further refinement to the methods discussed can be made to simplify and ease operations. Consequently, an improvement to the test precision may also result. Of particular, fine-tuning of methods is suggested for:

a) Gas volume measurement

Both continuous and batch (tube and bottle) reactors involves gas collection externally and emptied into a gasometer for volume determination. These external containers are cleaned and vacuumed each time before reattached to the reactors for more gas collection. Sometimes, if gas is being produced in high volumes and at a shorter rate, one bag or balloon is insufficient. This requires regular monitoring in case extra containers need to be added, which could be cumbersome especially in the weekends.

Therefore, automation to the gas measurement method is sought. This could be as simple as equipping a water displacement device with a sensor, a 3 way valve and a digital counter. An example of such a device can be found in the research of (Guendoz et al., 2007), where a set amount of displaced liquid when reached, activates the sensor which then regulates the valve to re-set the liquid level and release the accumulated gas. At the same time, the counter ticks to mark the volume of biogas measured. A simpler device using a pressure transducer can also be set using the same concept. However, instead of measuring the volume displaced, the device measures the pressure increase as equivalent to the volume produced. An example of this gas measuring system can be found in the work by Angelidaki et al. (1998).

b) Continuous reactor configurations

Two of the obvious difficulties with the continuous reactors operation were the manual mixing as well as the manual feed and waste technique. Due to the budget constraints, more sophisticated means were not feasible. However, should a further study be carried out, it is highly recommended that a mechanical system for feeding/wasting should be included in the research budgeting. As per (a) above, an automated system for the reactor stirring and feed/waste mechanism are suggested as this can help reduce the time spent and researcher fatigue.

CHAPTER 4

TUBE DESIGN AND DEVELOPMENT

4.1 INTRODUCTION

The test typically used to assess anaerobic biodegradability of liquid samples involves substrates being incubated along with seed, nutrient and buffers in 250 ml to 5 L bottles (Owen et al., 1979; Bogner, 1990; and Wang et al., 1994). The methane (CH_4) contribution resulting from sample decomposition is obtained by subtracting the background values (seed blanks) from the total. These tests can be of limited value for many solid samples, eg food wastes (FW), municipal solid waste (MSW), current or historic landfilled solid waste etc. One concern is the need to modify the solid sample prior to testing. Most test methods involve drying, grinding, re-drying and re-grinding to 2 mm or less (Nopharatana et al., 2007; and Gunaseelan, 2004). These modifications make the test results difficult to apply to field conditions. In addition, the steps involved in sample preparation could lead to oxygen exposure, which could distort the results. Finally, because of a small sample size of about 10-50 g w/w, the test result may not be representative of the bulk material. This research is looking at a new method that will enable analysis on larger sample sizes that would eventually cater for a variety of solid organic waste and conditions. The upgrading of the current technique of determining anaerobic degradation is valuable since batch bioassays are being used for planning large scale waste treatment plants.

4.2 METHODOLOGY

Details on the substrate, batch test reactor, seed and analytical techniques are provided earlier in the Methodology Chapter. Only the experimental procedures specific to the results of this chapter are discussed here. The description is divided into four sections, concerning the (i) seed (ii) substrate (iii) reproducibility issue and (iv) establishment of the tube operating procedure.

4.2.1 Seed

To study the effect of seed on the methane production of food waste, particular attention will be paid to the source of the seed, its volume, feasibility of seed thickening and seed pre-digestion.

4.2.1.1 Seed source

Seed from a laboratory stirred tank reactor operating at 5 g VS/L. d on food waste and 30 days hydraulic retention time (HRT) was compared to digested sewage sludge (DSS) from a local wastewater treatment plant (WWTP). At the time of the experiment, the food waste reactor had been operating for 190 days and it was assumed acclimatisation of the seed organisms to the food waste had occurred. Acclimatisation is defined following a stable effluent quality (VS, ammonia and pH levels) as well as steady gas production. For each of the seed sources, a pair of tubes was prepared where one was mixed hourly while the other mixed once daily. All the tubes were loaded with 1.5 L of the respective seed and 120 g of food waste. A seed blank for each of the different sources was prepared at 1.5 L without any substrate addition. The DSS contained 1.3 g VS/L while 2.7 g VS/L was present in the laboratory grown seed.

4.2.1.2 Feasibility of seed thickening

Seed thickening has the potential to produce seed with more rapid degradation of substrate per volume, lowering the seed:substrate ratio, and thereby increasing the substrate sample size that can be tested. If the water can be separated from the solids, a higher total solids (TS) and volatile solids (VS) concentration from the different separated layers is hypothesised. For this, the collected DSS was allowed to separate out via gravity settling in a

500 ml glass cylinder without mixing for 3 hours. The resulting middle watery portion or ‘supernatant’ and the settled bottom sludge termed ‘concentrate’, were then added to an amount of food waste and incubated for 10-24 days. The top ‘scum’ layer was not tested given the small volume (< 50 ml) formed, which was inadequate and if used could lead to process souring. This test was repeated twice. The first trial had a similar food waste content of 20 g and varying fractions volume. The second had similar seed volume but varying food waste content of 20, 30 and 50 g wet weight. The test conditions are shown in Table 4.1.

Table 4.1 The description of tubes tested with varying seed fractions and substrate loading

Tube Label		St (ml)	Ct (ml)	O (ml)	S (g)
Test 1	ST500	500			20
	CT100		100		20
	CT50		50		20
	CT250		250		20
	O500			500	20
	Seed blank			500	0
Test 2	ST250	250			20
	CT250		250		20
	O250			250	20
	CT250		250		50
	O250			250	30
	Seed blank			500	0

St: Supernatant; Ct: Concentrate; O: Original; S: Substrate

4.2.1.3 Feasibility of DSS pre-digestion

To investigate the feasibility of pre-digesting the DSS before use in the batch test, the collected DSS was stored in the laboratory under strict anaerobic conditions without substrate or mixing at 35°C. After a storage period of 1, 4 or 7 days, 1.5 L of the pre-digested seed was then added to 120 g FW and incubated for 6 days to compare the performance of the various aged seed on methane yield of the substrate. This test is then repeated with a storage period of up to 14 days.

4.2.2 Substrate

It is of special interest to check the applicability of the tube against substrates of varying biodegradability and methane potential. One of the concerns would be to see whether the operating procedure subscribed for one substrate (e.g. incubation condition, substrate loading etc.) would hold or have to be changed to accommodate another substrate of different degradability. Therefore, apart from a mixed food waste, rice, cellulose, starch and glucose were also investigated. The food waste was tested at organic loading rates (OLR) of 4.7, 9.41, 14.1, 18.8, 25.1, 31.2 and 37.6 g VS/L. This corresponds to food waste amounts (in g wet weight) of 30, 60, 90, 120, 160, 200 and 240, respectively in a 1.5 L of DSS seed. The rice was tested at 6.8, 10.2, 11.3, 17.0, 22.7, 30.6, 37.4 and 45.3 g VS/L. For the others (cellulose, starch and glucose), an organic loading rate of about 6, 12 and 18 g VS/L were examined. An example for an organic loading rate calculation of food waste for 120g food waste fed into 1.5 L seed in the batch tubes is given below. Food waste contains 28% TS, 84% VS/TS (values from Table 3.1).

Organic loading rate, OLR (g VS/L) = (Q * S)/ V

where Q: substrate flow rate (g/d), 120 g d^{-1}

S: substrate concentration in inflow (VS), 28% TS, 84% VS/TS

V: reactor volume (L), 1.5 L

Organic loading rate, OLR (g VS/L) = (120 g. d^{-1} * 0.84 VS/TS * 0.28 TS)/ 1.5 L

= <u>18.8 g VS/L</u>

4.2.3 Reproducibility Issue

The reproducibility of the tubes was checked via three means:

(i) testing 10 replicates of food wastes at 18.8 g VS/L. d simultaneously in the tubes,

(ii) testing triplicate of rice and food waste at an OLR 75% and 50% of the maximum OLR. For food waste this corresponds to 18.8 g VS/L. d (120 g w/w) and 12.6 g VS/L. d (80 g w/w), respectively when the maximum OLR is 25.1 g VS/L. d (160 g w/w). For rice this was 10.2 g VS/L. d (45 g w/w) and 6.8 g VS/L. d. (30 g w/w), respectively with a maximum OLR at 13.6 g VS/L. d (60 g w/w). The maximum OLR is defined as the highest amount one substrate can be tested without exhibiting a soured pH (<6.5) at the end of the incubation period. At the highest OLR, the methane yield may not necessarily be the highest value achievable. The maximum OLR threshold is estimated with 90% confidence.

The reason rice was tested was to check if the variance of results was solely attributed to the heterogeneity of the sample. Rice was more representative of a food waste sample than using a pure chemical like starch etc., while still having homogeneity and uniformity. Another intention was to check whether OLR has any effect on the issue of reproducibility, thus the testing of 75% and 50% of the maximum OLR.

(iii) seed blanks from various runs using the tubes were compared to check their performance in the same run and between runs. In other words, the point is to investigate if the repeatability and reproducibility of results could also be a factor in seed performance.

4.2.4 Assessment of Total Anaerobic Biodegrability

4.2.4.1 Theoretical methane potential (TMP)

The food waste sample was sent to two labs; Massey University for the chemical composition and Hill Laboratories for the total carbon, nitrogen and phosphorous. These values are then used to determine the C/N ratio and the theoretical methane potential based on Buswell's formula (Angelidaki and Sanders, 2004):

$$C_nH_aO_b + (n\text{-}a/4\text{-}b/2)\ H_2O \longrightarrow (n/2+a/8-b/4)\ CH_4 + (n/2-a/8+b/4)\ CO_2$$

Results from various runs on 18.8 g VS/L. d of food waste in the tubes were compared with the theoretical value. A pattern was looked for during the incubation period for methane yield that is maximum and close to the theoretical value as well as others that may indicate a sufficient degree of biodegradation within a shorter time. This can be exampled by Figure 4.1 where the horizontal dotted line indicates the theoretical methane potential, while the two vertical dotted lines represents two conditions (a) the maximum methane yield (may be >95% of theoretical value) and (b) at a lower methane yield (probably 50-75% of theoretical value). From this, a suitable experimental time may be suggested.

4.2.4.2 Mixing

The experiment on mixing effects was performed two times to confirm its significance. Two mixing modes were trialled; once per day (before gas measurements) and hourly for 14 hours straight starting from 7 am ending 9 pm. The mixing method is similar where the tube is shaken horizontally for 20 seconds. The tests were run for up to 26 days. The first trial had only single set-ups, while for the latter, triplicate was used. All set-ups include 120 g food waste in 1.5 L DSS (OLR ~18 g VS/L. d) and 1.5 L seed blank.

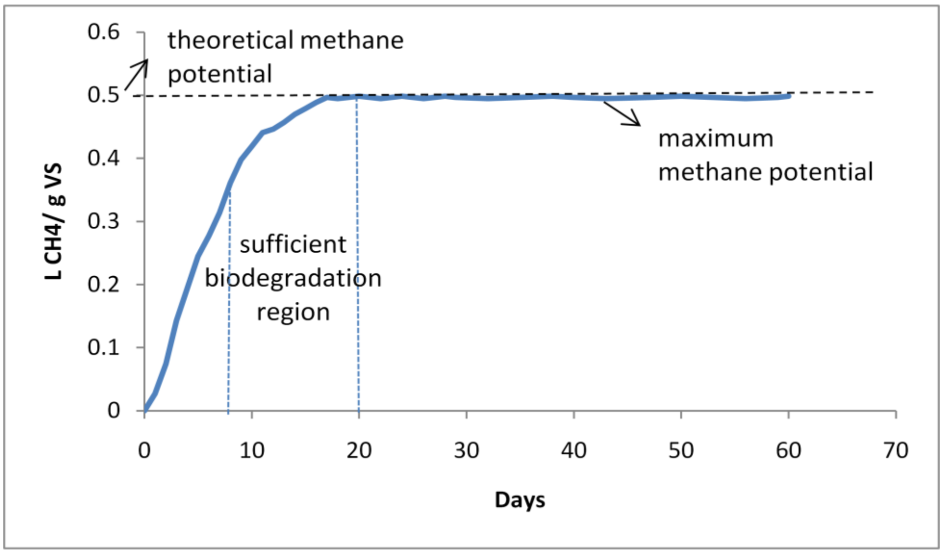


Figure 4.1 Illustration of biodegradation stages and methane generation

4.2.4.3 Trace elements

Two trials were run for the purpose of investigating the effects of trace elements and buffer supplementation. The first trial involved of 15 ml trace element solution being added to triplicates of 160 g food waste with another set of triplicates having 150 ml buffer solution added to it.

4.2.4.4 Buffer

In the second trial, three different buffer amounts were tested, 50 ml, 100 ml and 150 ml. The buffers were added to 160 g substrate and 1.5 L seed. The buffer solution was 50 g/l phosphate buffer while the trace elements used were as Table 3.4. The recipe for the trace elements was according to the one used in the laboratory of an environmental biotechnology consultant in New Zealand known as Waste Solutions (Chapman, 2006). This recipe is not far from the trace element solution used in the research by Raposo et al. (2006) and Zhang et al. (2003).

4.2.4.5 Reactor headspace

To investigate the effect of reactor headspace on the methane potential of a test substrate, two reactor configurations were checked. In one configuration, 120g food waste was loaded with 1.5 L DSS, into the 3.4 L tube, leaving a 56% void reactor headspace. In another, only 12% void reactor headspace was allowed, doubling the food waste and DSS content to 240 g and 3 L, respectively. For both of the configurations, the substrate and OLR was similar, comprising of food waste at 18.8 g VS/L. d. OLR. The purpose of conducting such a test was to check whether increasing the sample volume, maximising the reactor space and reducing the void volume would be beneficial towards improving the test precision and/or its reproducibility.

4.3 RESULTS

4.3.1 Anaerobic Respirometer Development

4.3.1.1 Design and procedure

The developed anaerobic respirometer as shown in Figure 4.2 and 4.3 is made of 10 cm diameter polyvinyl chloride (PVC) pipe measuring 41 cm long with 3400 ml capacity. The endcap at the bottom is fixed while the other is screw cap for easy sample introduction. The screw cap at the top was drilled to make connections for valves for sampling and gas collection. The blue tubing which runs off the gas analyzer (GA 2000 Plus, Geotechnical Instruments, UK) allows reading of the gas composition of the tube headspace when it is connected to the valve of the tube. Figure 4.2 depicts the procedure for using the respirometer to measure anaerobic decomposition from a solid sample. The following example used 120 g of pre-prepared food waste as the test substrate. For analysis and monitoring, steps as shown in Figure 4.3 were used.

4.3.1.2 Establishing food waste loading

The first step in conducting an analysis of methane potential and anaerobic decomposition of a given substrate is establishing a suitable substrate loading. Food waste when tested by itself without the addition of seed barely produced a measurable methane, as shown in Figure 4.4. After incubating for 33 days, the highest obtainable methane out of a set of triplicate is 0.0008 L CH_4/g VS. This is not surprising since at the start, the food waste had an average pH of 4.8. The substrate remained acidic with an average pH of 5.5 after the test completion. Clearly, a batch methane potential test will benefit greatly with some addition of seed to provide the necessary bacteria, thus cutting the experimental time significantly.

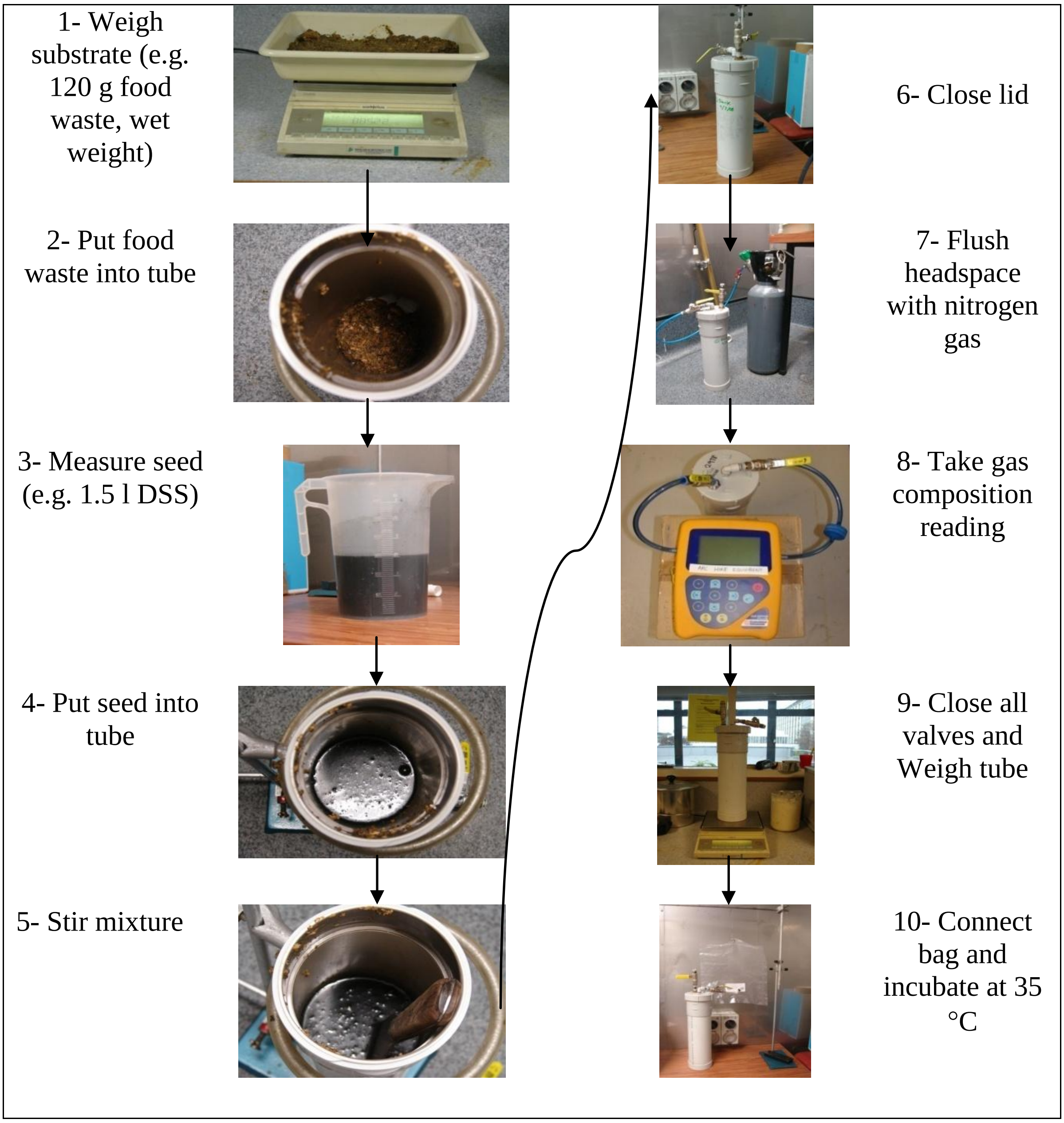


Figure 4.2 The tube design and operating procedure that was developed for measuring anaerobic decomposition of solid organic samples

Step 1 Shake tube horizontally for 40 seconds daily	
Step 2 Measure gas composition using gas analyzer	
Step 3 Measure gas volume by pressing biogas collected in Tedlar Bags into gasometer	

Figure 4.3 Techniques to determine tube gas production

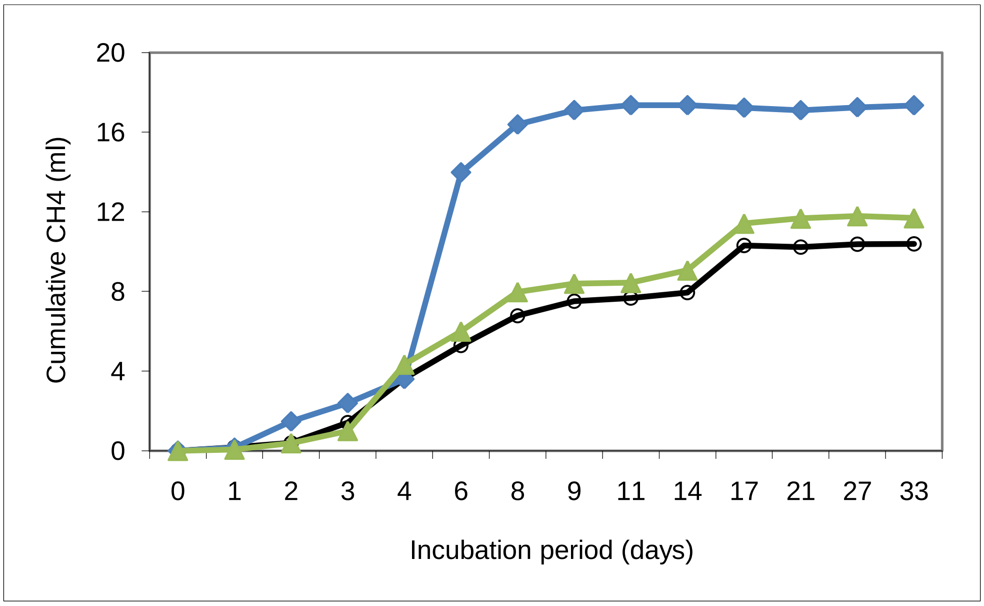


Figure 4.4 Accumulated methane production from food waste without the addition of seed or any supplements

To help establish the right substrate loading, the term OLR is used, which defines the amount of substrate that can be loaded into a certain reactor volume. The reactor volume usually consists of seed and may include extra nutrients and buffer. Food waste for example has been tested with different loading into the tube as shown in Figure 4.5. As can be seen, after 6, 20 and 34 days, the methane yield increases with the increase in loading rates. At 20 days, for example, methane yield increased from 0.265 $\pm$ 0.02 L CH_4/g VS (9% C.V.) at 9.4 g VS/L OLR to a yield of 0.313 $\pm$ 0.12 L CH_4/g VS (40% C.V.) when OLR was elevated to 18.8 g VS/L. However, a higher loading rate of 31.2 and 37.6 g VS/L, was not possible since the reactors had soured to a pH of 5.5 and 5.7 respectively, (refer Table 4.2) after just 6 days incubation.

It is therefore established that for use with the tube, a maximum organic loading of 25.1 g VS/L. d is tolerable for food waste. Even so, an OLR of 18.8 g VS/L. d is suggested when operating the tube as a higher methane yield can be achieved and the process is more stable.

Figure 4.6 and 4.7 show the hydrogen gas evolution with the 18.8 OLR and 25.1 OLR food waste. The former produced a higher hydrogen at 31000 ppm compared to 8000 ppm with the latter loading rate. It can be seen that for both loading rates, the hydrogen was highest at the start of the incubation e.g. after two days which decreases and remained less than 30 ppm thereafter until the end of the 22 days test. The hydrogen progression conforms to a healthy anaerobic digestion process. This means the hydrogen and carbon dioxide would be highest early in the experiment due to the hydrolysis of insoluble organic polymers to simple sugars. The acidogenesis process that follows convert the organic acids formed in the hydrolysis and fermentation stage to acetic acid, thereby decreasing the carbon dioxide and hydrogen concentrations. This explains the same drop in hydrogen gas seen with the 18.8 and 25.1 OLR food waste digestion.

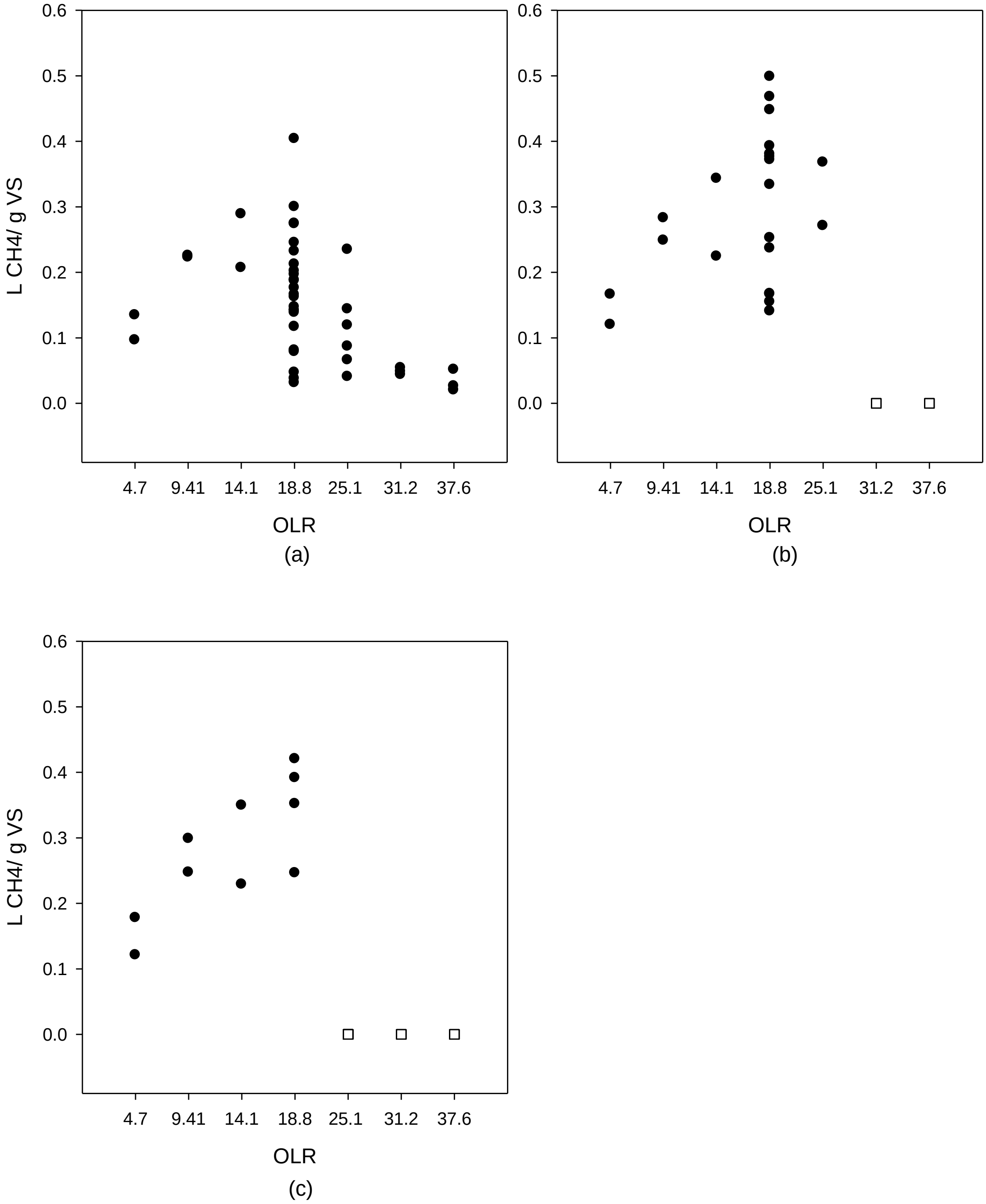


Figure 4.5 The effect of organic loading rates of food waste on methane yields after (a) 6 days, (b) 20 days and (c) 34 days. The white square symbols represent reactors that have soured

Table 4.2 pH of food waste effluents at completion

OLR (g VS/L. d)	pH	Day taken
4.70	7.4	34
9.41	7.4	34
14.1	7.4	34
18.8	7.4	20
25.1	7.5	20
31.2	5.5	6
37.6	5.7	6

The reduced methane yield for the higher OLRs suggests that the methanogen population could be a limiting factor. This is hypothesised based on the observation that the increasing substrate loading should have generated an increasing methane yield. Instead, there seems to be a breaking point in the loading rate where the methane yield dropped, indicating the amount of bacteria is insufficient to utilise the substrate. Furthermore, the acidic pH with the 31.2 and 37.6 OLR signifies that overloading has occurred. Instead, for the lower OLRs, substrate could be limiting which resulted in the lower methane yields. For the latter assumption, the influence of substrate loading on the methane yield will be validated against other substrates.

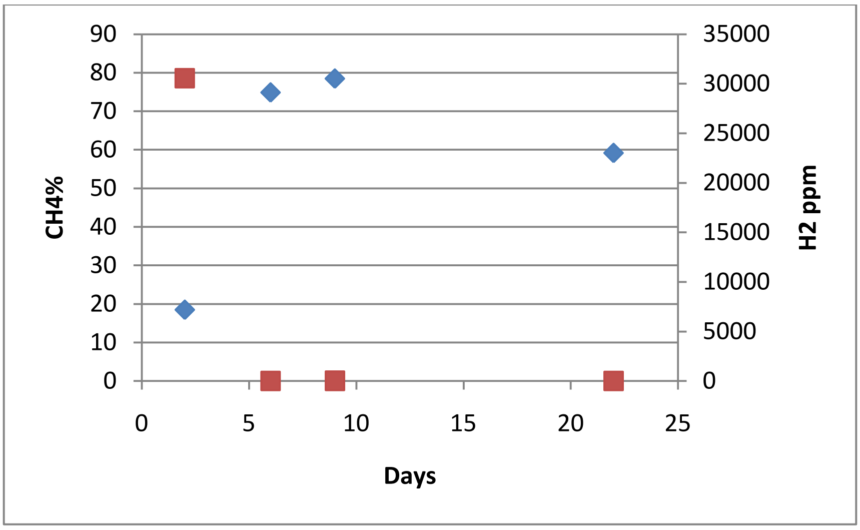


Figure 4.6 Hydrogen progression against incubation time and methane percentage for food waste digestion at 18.8 OLR. The square symbol represents H_2 while the diamond symbolises the CH_4%

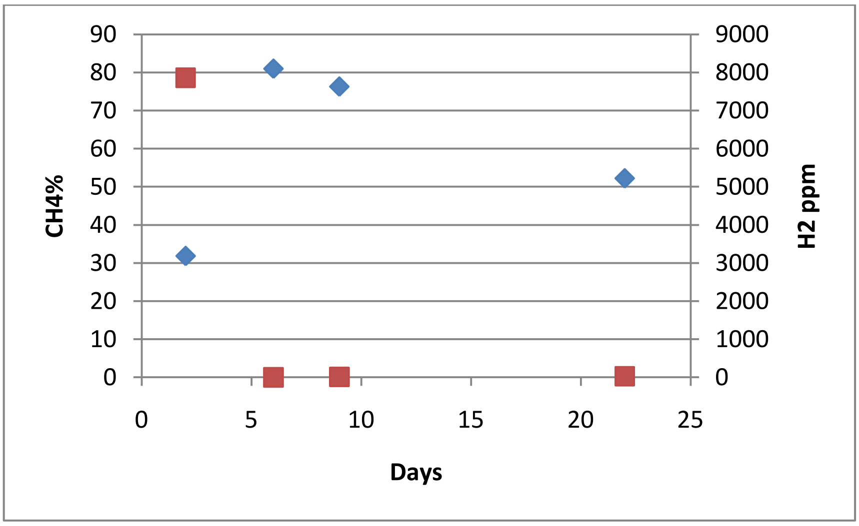


Figure 4.7 Hydrogen progression against incubation time and methane percentage for food waste digestion at 25.1 OLR. The square symbol represents H_2 while the diamond symbolises the CH_4%

4.3.2 Seed Quality

4.3.2.1 Seed source

Two different seed sources (i) digested sewage sludge (DSS) from a local wastewater treatment plant and (ii) effluent from a 190 days laboratory operated reactor were compared. Although the VS of the laboratory grown seed was two times that of the DSS it is anticipated that the varied VS contents between the two seed would not affect the methane potential result of the test substrate. This is because the reported values have already excluded the methane from seed blanks, meaning that only the methane from the substrate is considered and not methane contributed by the seed. The results shown by Figure 4.8 demonstrated that a higher methane yield was achieved by using DSS instead of seed from a master culture. The reactor seed used in food waste digestion produced only 72% of the methane yield obtained by using DSS which was 0.347 L CH_4/g VS. The DSS use in a similar test generated a higher methane yield of 0.448 L CH_4/g VS, further confirming the suitability of DSS as inoculum for the tube. From this point onwards, experiments with the tube were performed using DSS as seed.

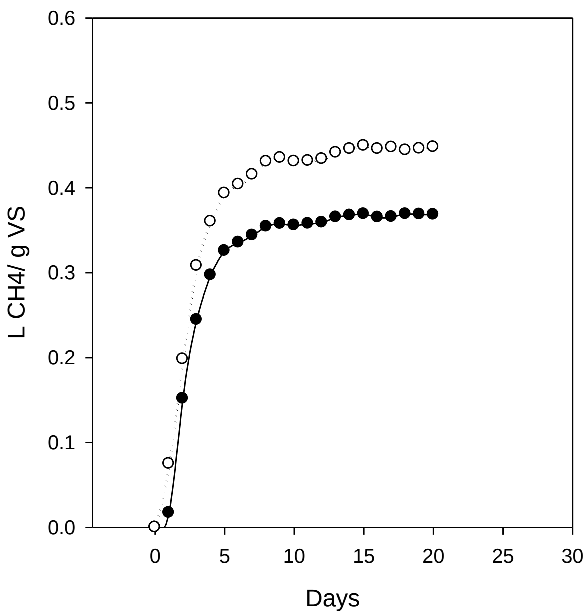


Figure 4.8 Methane yields of food waste using seed sourced from a laboratory operated reactor (bold circles) and from a wastewater treatment plant (white circles).

4.3.2.2 Seed thickening

There may be opportunities to reduce the volume of sewage sludge used as seed by a simple, preliminary treatment. Digested sewage sludge when left to settle typically separates out to three noticeable layers: a thin floating scum layer, a watery middle layer followed by a bottom sludge layer. The argument was whether the same methane yield can be extracted by using a more concentrated seed.

After gravity settling for 3 hours, the DSS separates to two major layers; a 250 ml supernatant and 250 ml concentrated sludge. The supernatant and concentrate layers had a total solids content of 0-0.3% and 2-2.4% respectively, of which an average 68% and 72.5% were volatile. The initial DSS values had 1% TS and 70% VS/TS. Gravity settling thickened the seed by 2.4 times. When these different layers inoculated a similar amount of substrate, a higher methane production from using sludge in its unaltered form results (refer Figure 4.9). This observation was true on both tests, either 500 ml in test 1 and halved to 250 ml in Test 2.

The use of concentrate (250 ml) in both Tests 1 and 2 reached nearly 90% of the methane percentage potential obtained by the original DSS. The methane percentage declined substantially when supernatant was added to the substrate, giving only half of the methane percentage in Test 2. In Test 1 however, the methane percentage was fairly comparable with the other seed conditions but the supernatant volume was twice that of the concentrate. The findings in Figure 4.9 indicate that methanogens are present throughout the seed, more probably attached to the seed particles than its liquid form, but not enough to justify regular concentration of the sludge.

In Test 1, the methane percentages plummeted in conjunction with decreasing concentrate volume from 250 ml to 100 and 50 ml and were in fact lower than the blank. Ph of effluents at completion (after 24 days) was 6.8 and 6.7, respectively for the 100 ml and 50 ml concentrate test conditions. On a similar scale, when more substrate was added, 30 g for the original (unaltered) DSS and 50 g for the concentrate (refer Test 2), only 6% and 28 % CH_4

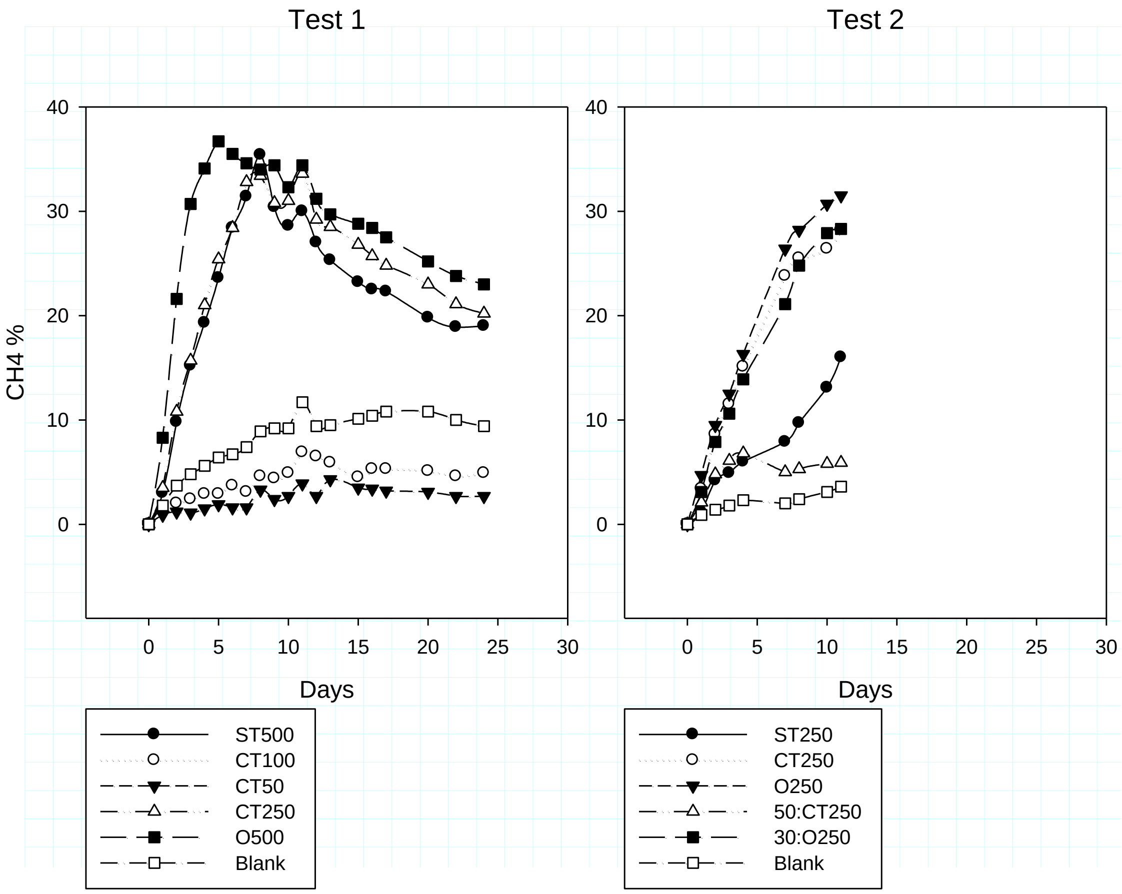


Figure 4.9 Effect of different seed layers on methane percentage of food waste digestion. All tubes were loaded with 20 g FW except in Test 2 which had 50 and 30 g substrate (wet weight), labelled 50:CT250 and 30:O250, respectively. The terms ST, CT and O represents the layers of seed used; supernatant, concentrate and original. The numbers e.g. 50, 100, 250 and 500 after the terms shows the volumes of respective layers added

respectively was produced. This was 79% less the methane percentage potential with 20 g substrate for the former and 11% less methane percentage potential for the latter. For the 50 g loading, the pH was an acidic 5.7, while neutral at 7.5 for the 30 g loading. It seems that trying to load more substrate from 20 g to 50 g when using concentrate is not viable. Thus the notion that microbes might be more populated in the concentrate (thus allowing more substrate in testing) may not be true as was initially thought to be. In fact, there stood a better chance at increasing the substrate loading (above 20 g) when an unaltered DSS containing both the liquid and solid form was used.

Figure 4.9 demonstrates that methanogens may be potentially limiting under circumstances like decreasing seed volume or increasing substrate addition. In light of this preliminary observation, possible factors limiting the anaerobic digestion of solid substrate are investigated and discussed in the Section "4.4.6 Reproducibility of results". Since no apparent advantage arises from thickening the DSS, it was decided to use the seed as it was sourced from the treatment plant in the following experiments.

4.3.2.3 Seed pre-digestion

The seed collected from the Christchurch Wastewater Treatment Plant has always been stored without mixing or substrate addition for seven days at 35°C before use in the batch tests. The effect from using stored seed instead of a fresh one in a batch test was investigated and results are presented in Figure 4.10. The variation in food waste methane production for both a duplicate (Test 1) and triplicate set (Test 2) was more pronounced for the 1 and 4 day predigested seed compared to using a 7 day predigested seed.

As shown by Figure 4.11, the variation in methane yields seems to be slightly more reproducible with an increased seed pre-digestion period. This observation is supported with the corresponding averages and standard deviations (in brackets) of food waste methane yields after 1, 4, 7 and 14 days predigested seed; 0.231 ($\pm$ 0.09), 0.243 ($\pm$ 0.07), 0.216 ($\pm$ 0.07), and 0.113 ($\pm$ 0.02). However, the 14 day pre-digested seed indicated a lower methane production, which could impede finding results quickly. It seems that some time is required for microbes in the 14 day pre-digested seed to reestablish their activity before being capable of processing the substrate at a faster rate. This may mean for a 4-7 days pre-digested seed it may take 20 days to estimate the cumulative methane yield whereas maybe a longer 27 days is required for a 14 days pre-digested seed to achieve the same cumulative methane yield.

The seed blanks of both Test 1 and 2 saw a greater background methane production from fresh seed compared to when the seed was pre-digested for 4 or 7 days which were roughly at par. As a result, it was decided that the 7 day predigested seed presented a high methane production from the substrate. A minimal amount of methane from the seed is preferred because it would not blur the result of methane production from the test material especially if low gas production is anticipated.

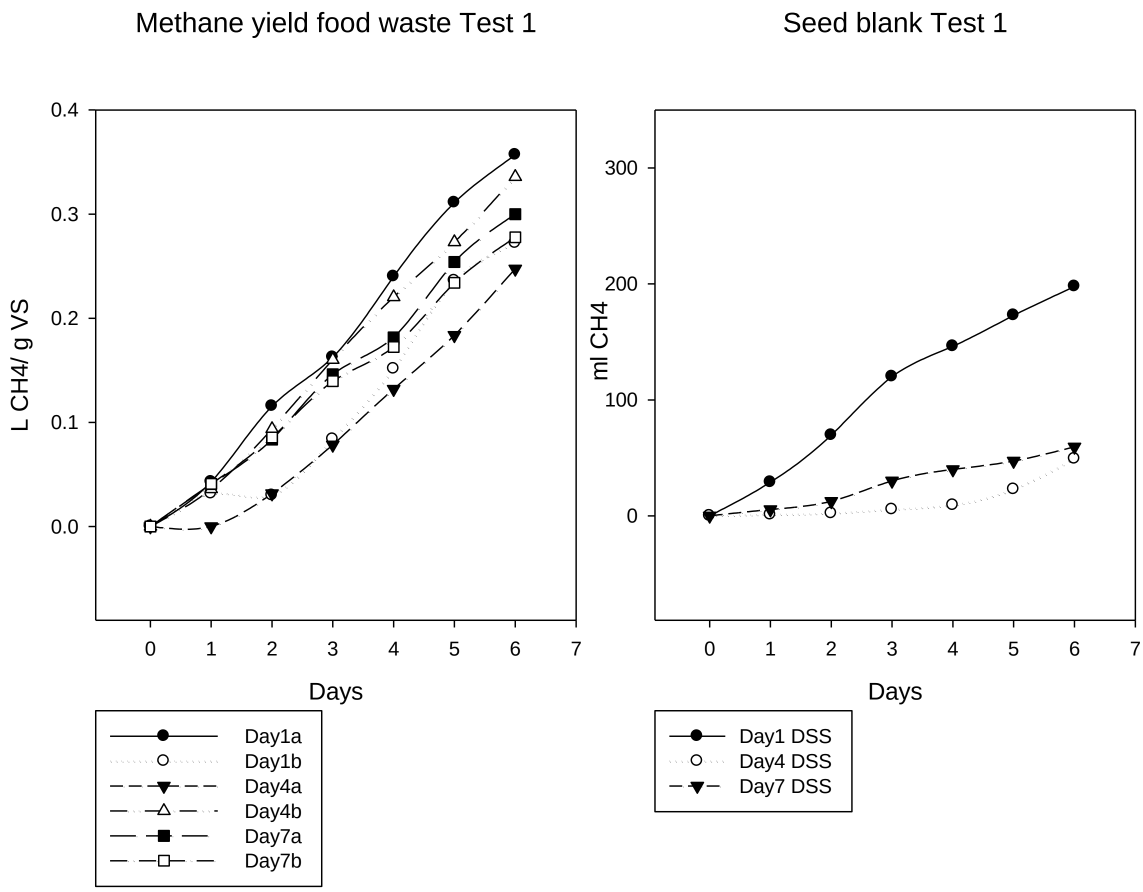
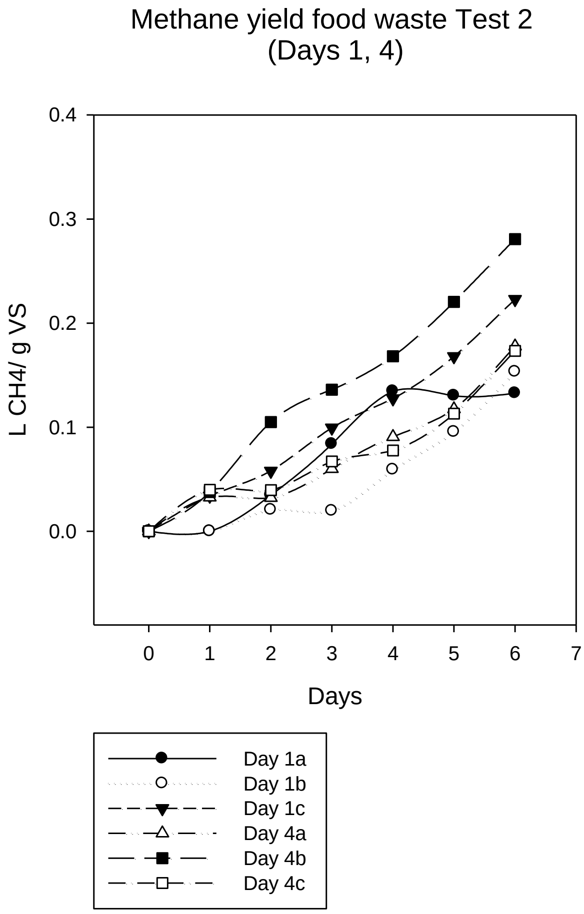
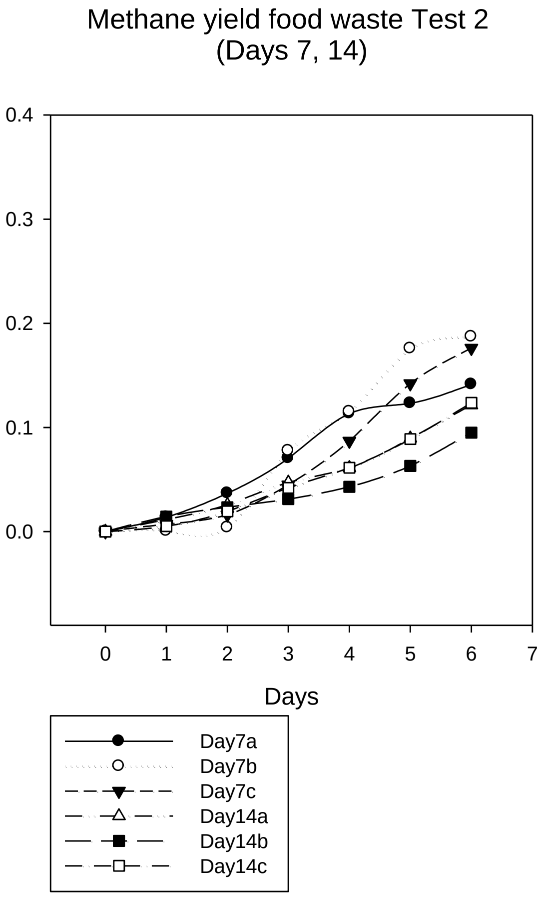


Figure 4.10 The methane yield of food waste from using 1, 4 and 14 days pre-digested seed in the batch test (7 day results are presented). Experiment was repeated twice with a 14 days investigation added to the latter. The methane yield of food waste considers only VS of food waste with the methane yield reported after subtracting off the average seed blank





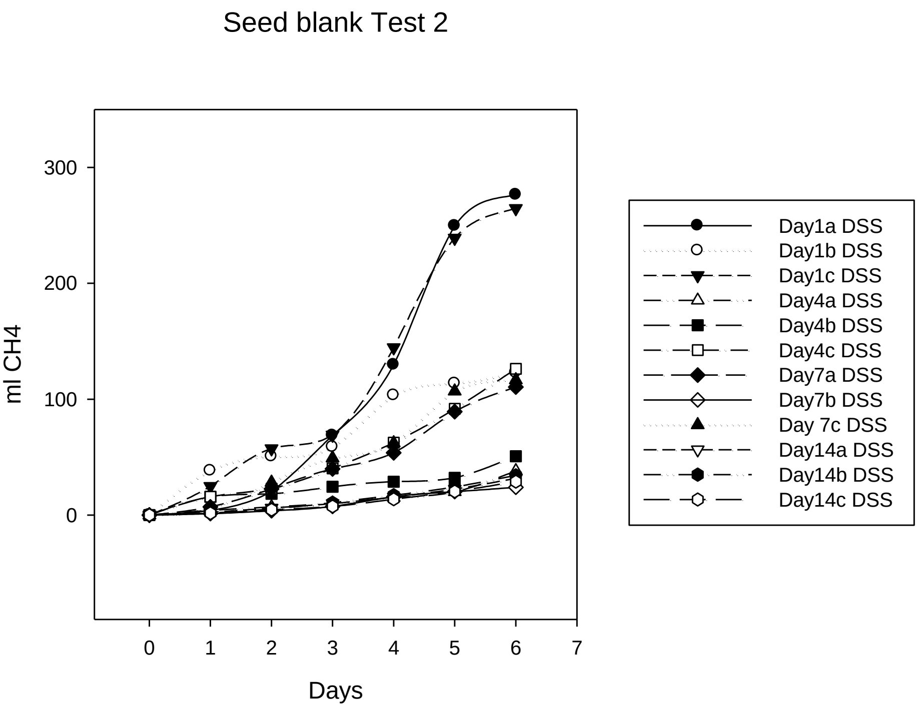


Figure 4.10 continued

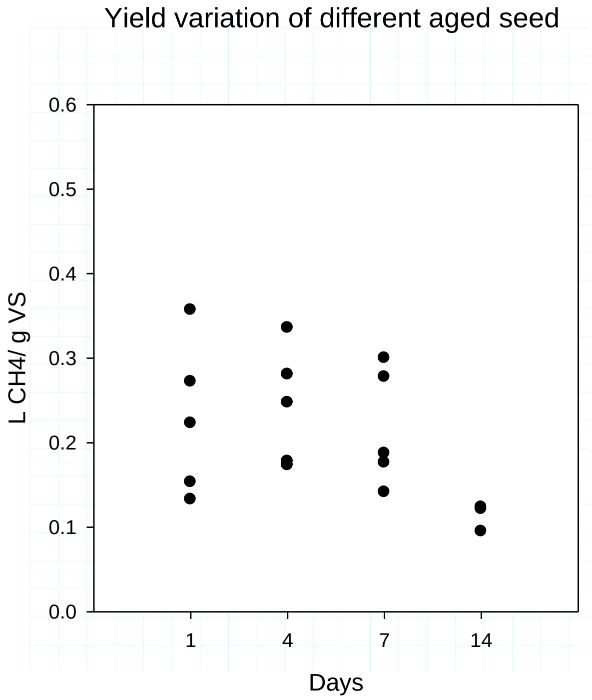


Figure 4.11 The effect of seed pre-digestion period on food waste methane production. The days on the x axis represents the pre-digestion period underwent by the DSS before use. The data reported are methane yield after 6 days incubation

4.3.3 Substrate Loading

4.3.3.1 Cellulose, starch and sucrose

When cellulose, starch and sucrose were evaluated at three loading rates; ~6, ~12 and ~18 g VS/ L. d., an interesting pattern was observed, referring to Figure 4.12. The highest methane yield of 0.619 and 0.574 L CH_4/g VS was achieved from sucrose and starch, respectively, each at the lowest loading rate of 6.7 and 6.2 g VS/L. d. Both substrates decreased in methane yield when the loading rate was raised to about 12 g VS/ L. d. The declination was greater for sucrose producing only 0.040 L CH_4/g VS than starch with a yield of 0.134 L CH_4/g VS. The same reaction is repeated when the loading rate was further increased to about 18 g VS/L. d. for both sucrose and starch. The effluent pH also decreased with increasing substrate loading, implying a more stable digestion occurring at lower organic loading rates for both sucrose and starch (refer Table 4.3).

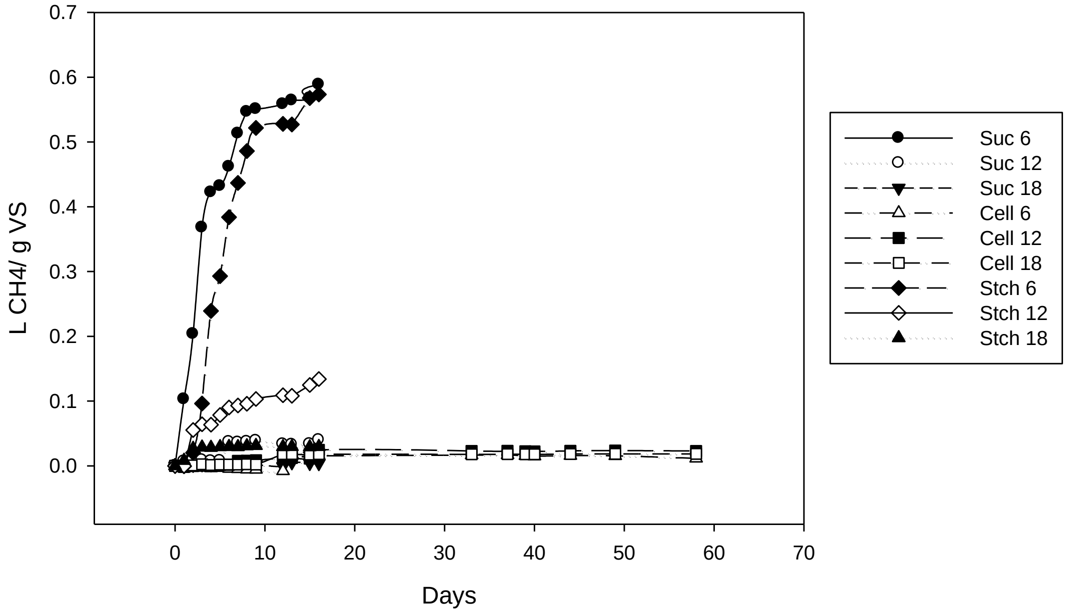


Figure 4.12 Methane yield in response to different substrate of varied biodegradability and organic loading rate. The terms Cell, Suc and Stch each represents cellulose, sucrose and starch. The number following that indicates the OLR

Table 4.3 pH of effluents at completion for sucrose, cellulose and starch with variable organic loading rates

Substrate	OLR (g VS/L. d)	pH	Day taken
Sucrose	6.67	7.3	16
	13.33	5.6	16
	20.00	4.1	16
Starch	6.20	7.4	16
	12.41	5.6	16
	18.61	4.9	16
Cellulose	6.14	7.6	58
	12.28	7.5	58
	18.42	7.4	58

Cellulose on the other hand, had a roughly equivalent pH value of 7.5 across the three different OLRs of 6, 12 and 18 g VS/L. d. The methane yield was also negligible for all the three OLRs as demonstrated in Figure 4.12, ranging from 0.01 – 0.02 L CH_4/g VS. Methane was barely produced as can be seen in Table 4.4, with a composition less than 30% at the end of the 58 days incubation. The fact that effluent pH is neutral indicates that anaerobic digestion has not soured or failed, but the low methane composition signifies that for some reason methane production was limited. It was later learnt that the type of cellulose used for the experiments ((MethoCel, Hydroxypropyl Methylcellulsoe, The Dow Chemical Co., USA) is used in the pharmaceutical industry as tablet coatings and thickeners to allow safer handling of potent drugs as well as producing desired viscosity in liquid medications. Because of their thickening and film forming properties, the cellulose compound is fairly resistant to microorganisms. This may have been the factor behind the limited methane production seen.

Table 4.4 Ratio of % methane/% carbon dioxide in the gas produced from cellulose degradation at varying OLRs

Days	6.1 g VS/L	12.3 g VS/L	18.4 g VS/L
1	8.0/8.6	10.3/7.6	9.8/6.9
7	21.5/16.2	24.5/20.0	24.1/23.2
13	25.4/20.6	32.1/25.4	21.5/26.5
33	27.0/17.1	20.9/37.1	27.6/22.5
44	24.6/19.4	23.8/34.3	25.6/24.4
58	21.2/22.9	27.5/30.5	28.8/21.2

The suitable organic loading rate for food waste has been established earlier to be 18.8 g VS/L. d. (refer Figure 4.5). It has to be remembered that food waste used here had low cellulose content (9% VS, refer Table 3.3). When utilising the food waste loading rate of 18.8 g VS/L. d to all three substrates; cellulose, starch and sucrose, methane was barely produced, highest being just 0.04 L CH4/ g VS for starch after 16 days (refer Figure 4.13). Only when the OLR was cut back by three times (~6 g VS/L. d) was a good methane yield (about 0.5 L CH_4/g VS) was achieved, sucrose being slightly higher than starch. Looking at Figure 4.13, the methane yield is affected by the substrate biodegradability as well as the organic loading rate. More explanations pertaining to Figure 4.13 is given under "Discussion" in Section 4.4.3.

4.3.3.2 Rice

Figure 4.14 depicts the methane production from rice (Sunrise Basmathi) across different organic loading rates including 6.8, 10.2, 11.3, 17.0, 22.7, 30.6, 37.4 and 45.3 g VS/L. d. The highest methane production observed in one of the four tubes tested at the lowest OLR (6.8 g VS/L. d) produced a methane yield of 0.542 L/g VS. This yield decreased by 33% to a maximum of 0.398 L CH_4/g VS in one of four tubes when the OLR was raised to 10.2 g VS/L. d. Further rise of organic loading saw the methane production dropping in parallel. This behaviour is clearly defined in Figure 4.15, where the methane production was observed to be dependent upon the substrate loading rate. The acidic effluent conditions between pH 4.1 to 5.3 recorded for the higher OLRs of 17.0, 22.7, 30.6, 37.4 and 45.3, verifies that the anaerobic process had soured when the substrate loading was pushed beyond 11.3 g VS/L. d. (refer Table 4.5).

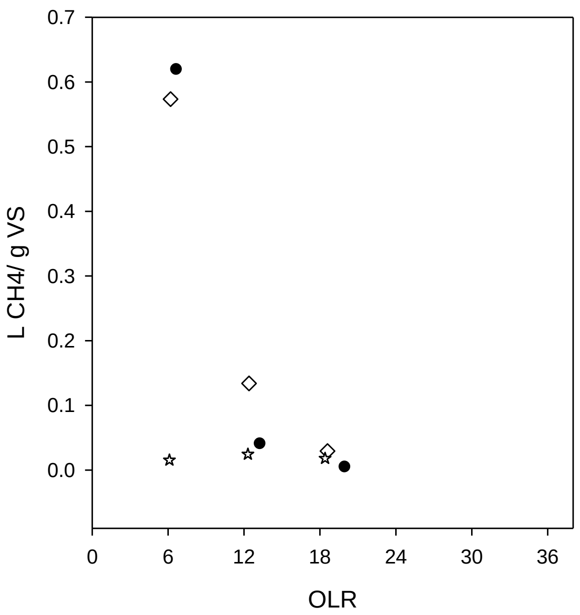


Figure 4.13 The effect of substrate biodegradability and organic loading rate to its methane production after 16 days incubation. The bold circle represents sucrose, diamond represents starch while the star symbolises cellulose

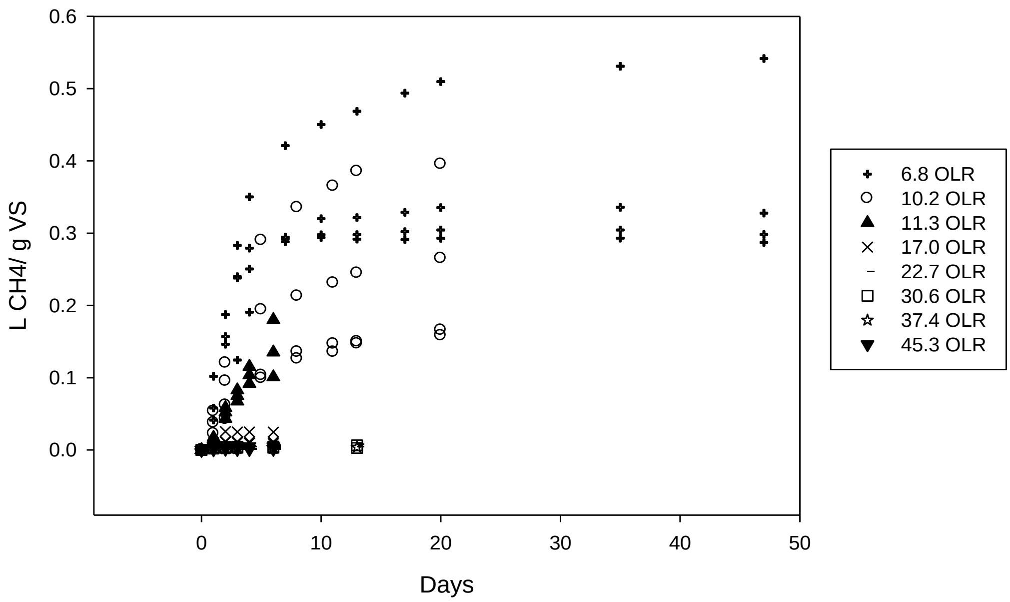


Figure 4.14 The methane yield of rice as a response to various OLR over time

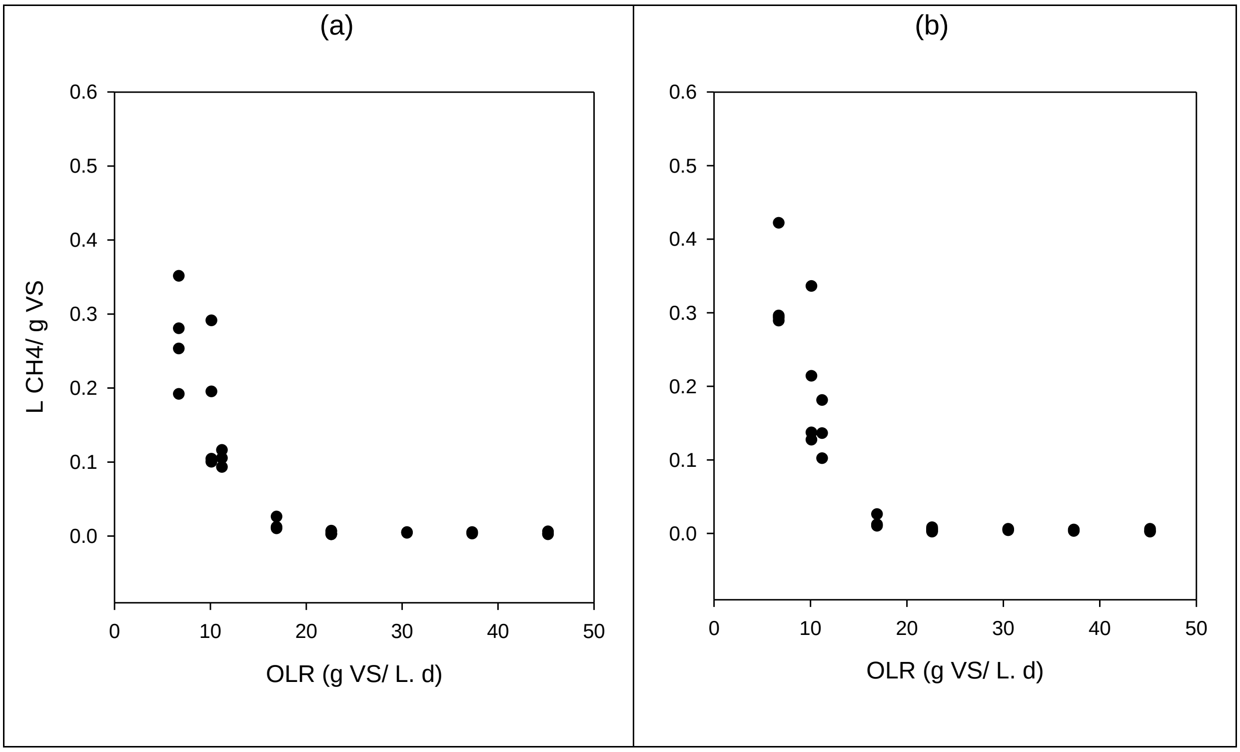


Figure 4.15 The effect of organic loading rates on methane production of rice after (a) 4 and (b) 6 days of incubation

Table 4.5 pH of effluents at completion for rice with various loading rates

OLR (g VS/L. d)	pH	Day taken
10.2	7.4[c]	20
11.3	7.4[b]	6
17.0	5.2[b]	6
22.7	5.3[b]	13
30.6	5.3[a]	13
37.4	4.1[a]	13
45.3	4.2[a]	13

[a] average of duplicate, [b] average of triplicate, [c] average of quadruplet

4.3.3.3 Comparison of yields between various substrates – cellulose, starch, sucrose, rice and food waste

As can be observed in Figure 4.16 (a), at an OLR of 6 g VS/L. d., the upper range of methane yield, 0.384 – 0.462 L CH_4/g VS was reported for sucrose, starch and rice. The corresponding pH was around 7.4, which signifies the 6 g VS/L. d OLR was suitable for biodegrading sucrose, starch and rice.

For most cases in Figure 4.16 (b) an unhealthy process, where the pH was acidic around 4.1 – 6.1, has been recorded across all substrates except cellulose. At these low pH, the methane yield for these substrate was limited, producing less than 0.12 L CH_4/g VS. A neutral pH (about 7.5) was also recorded for the same low yield of 0.12 L CH4/g VS for food waste and cellulose. The OLR for food waste at pH 7-7.5 was about 18 and 24 g VS/L, while cellulose was at 6, 12 and 18 g VS/L. The findings in this section not only highlights the possibility of varied tolerance to substrate loading and their effect on methane yield for different substrates, but further reinforces the importance of test replications in order to obtain a reliable and representative result.

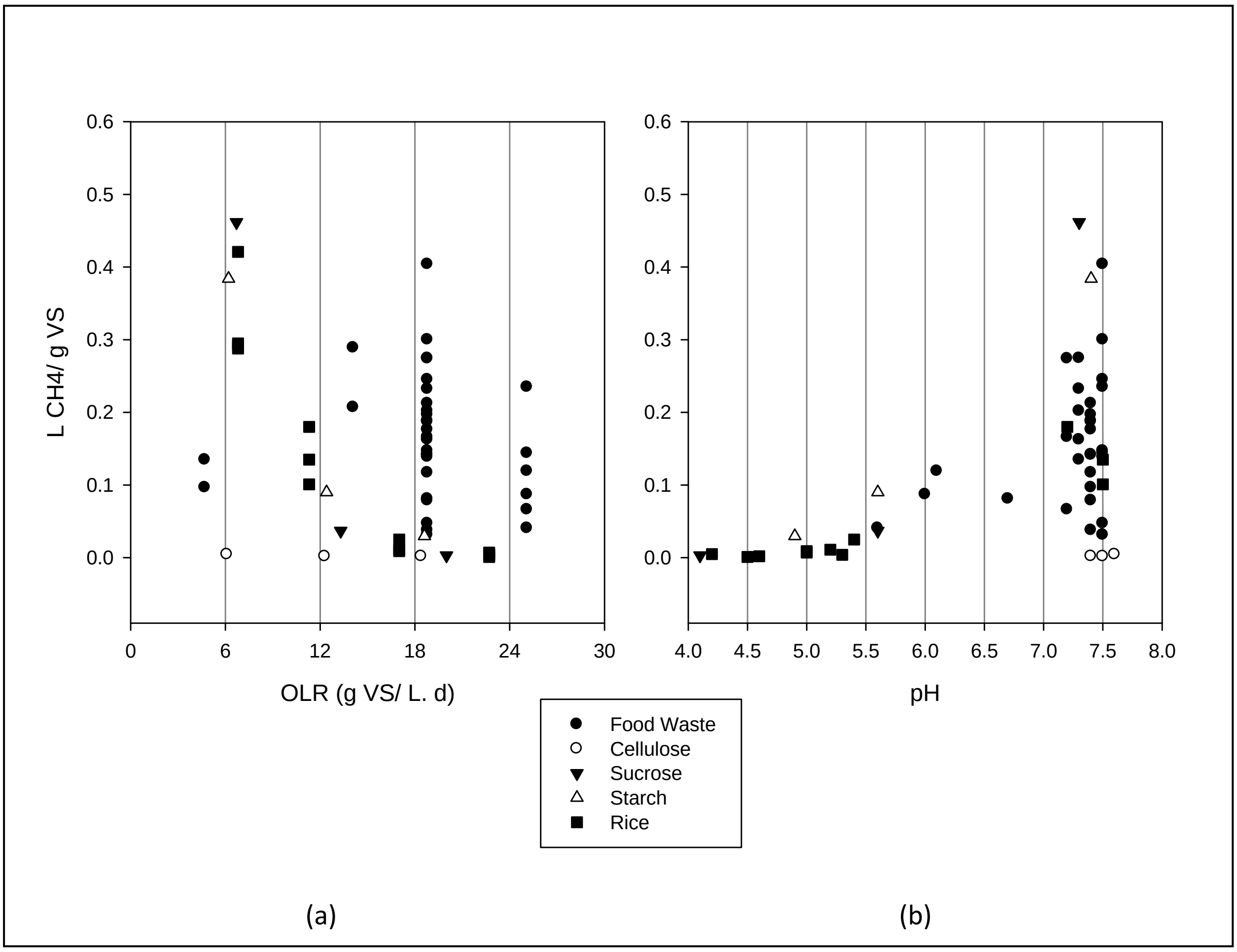


Figure 4.16 The methane yield in response to the (a) OLRs of different substrates after 6 days incubation and (b) effect on reactor pH

4.3.4 Reproducibility of Test Results

When 10 replicates of 18.8 OLR food waste were tested together in the tubes, large variations in methane yield were observed (refer Figure 4.17). Two causes for the irregularity were considered, one being the sample and the other, the seed.

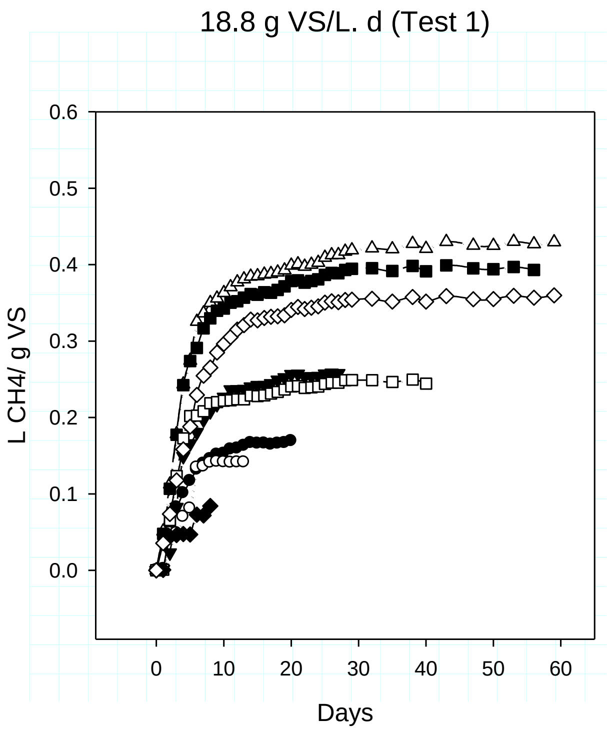


Figure 4.17 Methane yield from 10 replicates of food waste at 18.8 OLR conducted in one run

4.3.4.1 Substrate influence

Rice comprised of a more homogenous structure compared to food waste, thus was chosen to check the contribution of substrate to the irregularity of results. Figure 4.18 (b) demonstrates that rice gave no more reproducible result than food waste.

Variability seemed to increase with an increase in OLR for both substrates: food waste and rice. For example after 20 days incubation, for an OLR 75% of the max OLR, food waste had a methane yield average of 0.210 L CH_4/g VS and standard deviation of $\pm$0.11. The standard deviation decreased to $\pm$0.05 (average yield 0.293 L CH_4/g VS) when the loading was at 50% of the max OLR. The same was observed with rice which had a higher standard deviation of $\pm$0.11 for an average methane yield of 0.246 L CH_4/g VS at OLR 75% of the max OLR. When only 50% of the max OLR was tested, the standard deviation dropped to $\pm$0.04 for a yield average of 0.327 L CH_4/g VS.

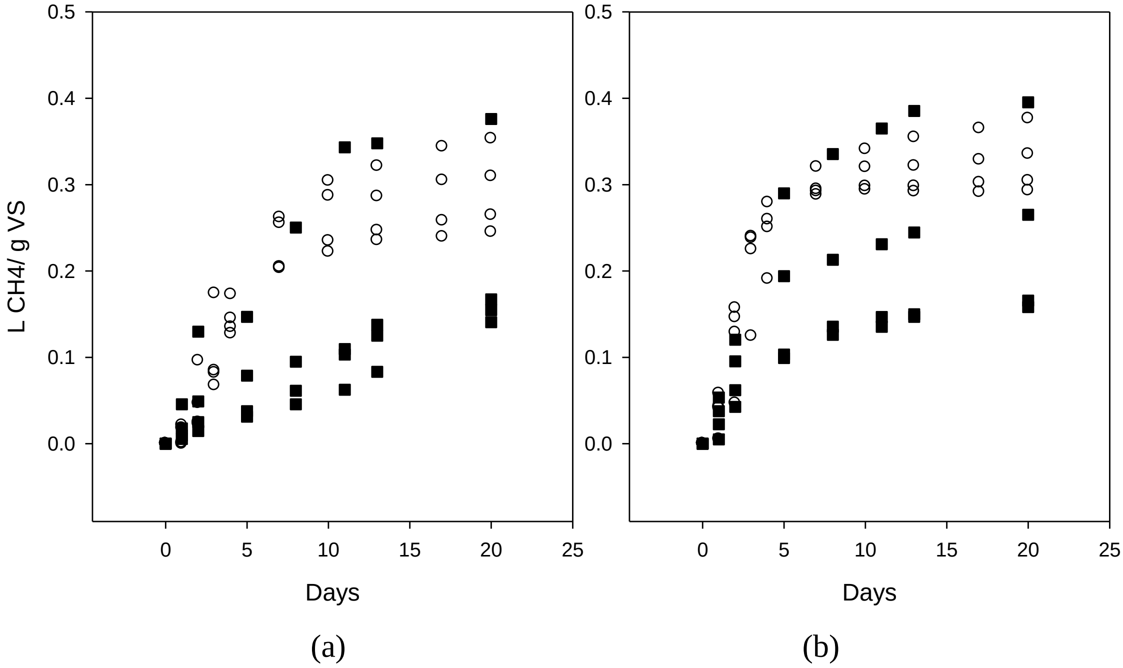


Figure 4.18 Methane yield of (a) food waste and (b) rice at OLR at 50% and 75% of the max. OLR. The bold square symbolises the 75% max OLR while the white circle represents 50% max OLR. The details of the maximum OLR estimation is given in Section 4.2.3

4.3.4.2 Seed influence

The seed at all times was digested sewage sludge (DSS) taken from the Christchurch WWTP 7 days before use. Upon collection, it was stored in the laboratory temperature control room at 35°C without feeding or mixing. A fixed volume of 1.5 L DSS was constantly added as seeding material in each tube for testing of food waste or rice. For each test run, a duplicate or triplicate DSS (1.5L from the same DSS batch) was provided as seed blank to provide information of the background biogas production.

Table 4.6 demonstrates a diverse response to within-run reproducibility of seed blanks used in food waste testing. For Test 1, 2, 3, 6 and 7 the variation between replicates in the same test was large, registering a coefficient of variation (CV) from 31.1 to 94.8 %. Nonetheless, there were also instances that the variation of replicates in a similar run was small (less than 23.7% CV), notably for Test 4, 5, 8 and 9. When the averages of these tests (Test 1-9) were compared together, a reasonable variability between-run was observed, having a coefficient of variation of 34.7 %.

Table 4.6 Reproducibility of seed blanks used in testing of food waste (observed after 7 days incubation)

	Average	Stdv	CV (%)	# Data
WITHIN-IN RUN VARIABILITY				
Test 1	32.6	10.1	31.1	3.0
Test 2	19.3	12.4	64.1	3.0
Test 3	83.7	51.8	61.9	3.0
Test 4	25.6	2.1	8.2	3.0
Test 5	48.8	11.6	23.7	2.0
Test 6	53.1	25.4	47.8	2.0
Test 7	37.4	35.4	94.8	2.0
Test 8	73.5	3.8	5.2	2.0
Test 9	48.8	11.6	23.7	2.0
BETWEEN-RUN VARIABILITY				
(Compares average of Test 1,2,3,4,5,6,7,8,9)	47.0	16.3	34.7	9.0

A similar response to replicate reproducibility seen above was also observed for seed blanks used in rice testing (refer Table 4.7). The duplicate seed blank in Test 8 was reproducible, having a coefficient of variation less than 5.2 %. However, the other two tests, Test 10 and 7, recorded a large variation between replicates with CV of 36.3-94.8 %. The between-run variability between these tests (Test 7, 8 and 10) was not much, registering a coefficient of variation of 27.5 %.

Table 4.7 Reproducibility of seed blanks used in testing of rice observed after 7 days incubation

	Average	Stdv	CV (%)	# Data
WITHIN-IN RUN VARIABILITY				
Test 7 trial 16*	37.4	35.4	94.8	2.0
Test 8 trial 20*	73.5	3.8	5.2	2.0
Test 10 trial 22	62.1	22.5	36.3	2.0
BETWEEN RUN VARIABILITY	57.7	15.9	27.5	3.0

*Note: results are similar to Table 4.9, seed blank was similar since food waste and rice testing was conducted in the same run.

Seed blanks that were reproducible consists both of duplicate (Test 5, 8, and 9) and triplicate (Test 4) sets, hence a duplicate seed blank is sufficed to be included for each test run. The methane generated from seed blank contributed to less than 10 % and 13 % of the total methane production from rice and food waste decomposition, respectively. Considering this small seed blank contribution, it may be possible to use only a universal seed blank value to be subtracted from all results, rather than using batch-specific seed blank values. However, this is only recommended after a large compilation of seed blank data is gathered and the methane contribution of seed blank is calculated. In addition, the universal seed blank value may only be used when substrate is tested at or below the optimum OLR. This is because the overall methane production tends to be limited the further tests are above the optimum OLR, which diminishes the methane produced from the seed in mixed seed/substrate tests.

4.3.5 Establishing Tube Operating Conditions

The chemical composition of the food waste sample used in the experiments as per Table 4.8 comprised of 3.4 % protein, 2.4 % fat and 8.3 % carbohydrate[1]. The theoretical methane potential of the food waste sample was calculated based on the Buswell's formula. Three values of the methane potential were obtained:

(i) 0.45 L CH_4/g VS using the known chemical fractions; protein ($C_5H_7NO_2$), fat ($C_{57}H_{104}O_6$) and carbohydrate ($C_6H_{10}O_5$),

(ii) 0.51 L CH_4/g VS based on maximal theoretical methane yield constant (350 ml CH4 (STP) g COD), and

(iii) 0.51 L CH_4/g VS based on the food waste elemental composition ($C_{5.73}H_{9.37}O_{3.67}$).

The calculations for these theoretical methane potential are appended in Appendix 4-a.

[1] Carbohydrate was estimated from these results = 100–protein–fat–moisture-ash-NDF= 100–3.4-2.4–76.5–4.5-4.9 = 8.3 % as received.

Table 4.8 Chemical composition of the food waste sample

Composition	DM	Ash	Fat	Protein	Carbohydrate	NDF	ADF	Lignin
Wet weight %	23.5	4.5	2.4	3.4	8.3	4.9	3.3	1.6
Dry weight %	23.5	19.2	10.3	14.7	35.4	20.9	14.0	6.9
% VS			12.6	17.9	43.6	25.8		

DM: Dry matter. Acid detergent fibre (ADF) is a measure of cellulose and lignin. Neutral detergent fibre (NDF) is a measure of total insoluble fibre and includes cellulose, lignin and hemicellulose. It is a better indicator of total fibre than ADF.

4.3.5.1 Experimental time

Figure 4.19 presents results from 18.8 OLR food waste replicates in the tubes which produced yields within 10% of the theoretical values of 0.45 L CH_4/g VS. The figure shows the methane production over time from 6 days until a maximum of 60 days incubation. It is clear that all replicates experienced the fastest rate of reaction between 0 till 6 days upon which it began to slow down. The rate of reaction seemed to level out and began stabilising from 20 days incubation. A close look at Figure 4.19 seems to highlight a slight burst of methane from 20 to 30 days of incubation especially with the 'X' duplicate. This burst in methane production is revealed further after examining its daily gas production presented by Figure 4.20. The methane production for the duplicate generally decreases each day from 20 until 30 days of incubation. Then the methane production increases daily (by about 2 – 4 times) until about the 35th day before again decreasing towards the end of incubation period. Although there was the small burst in the gas production especially between the 30th and 35th day of incubation, the effect to the total methane yield was insignificant. This is because such methane burst events was not common among various substrate incubation, and because even in this one case, methane contribution from this 'burst period' is less than 3% of the total methane production.

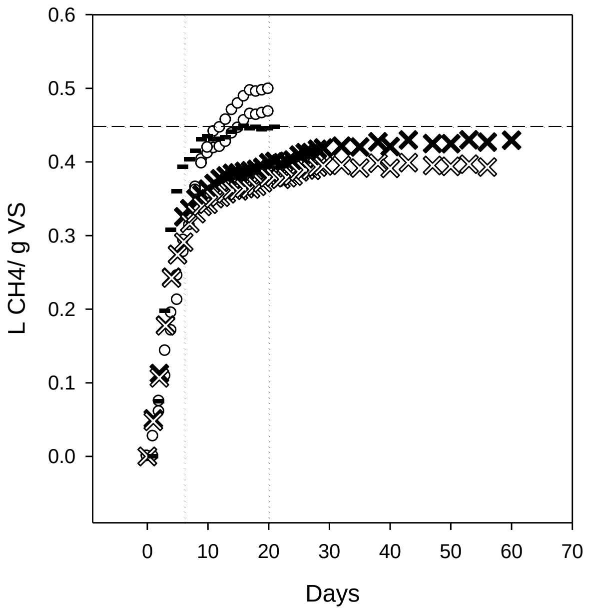


Figure 4.19 Methane production from 18.8 OLR of food waste over time. The bold and clear 'x' represents a duplicate in one test run. The white circles are duplicates in a run while a single replicate in another run is indicated by the black rectangle. The vertical dotted line outlines the theoretical methane potential for food waste. The vertical dots represent the methane yield achieved after 6 and 20 days incubation

One of the duplicate (bold 'X') (refer Figure 4.19) depicts a maximum methane yield of 0.429 L CH_4/g VS after 60 days. After 20 days incubation, the yield is 0.400 L CH_4/g VS equivalent to 93% of the 60 day yield while after 6 days recorded a yield of 0.326 L CH_4/g VS equivalent to 76% of the 60 day value. The other replicate (clear 'X') also behaved similarly. Methane yield obtained after 6 days was at 74% (0.291 L CH_4/g VS) of the 55 day methane value of 0.393 L CH_4/g VS. The result after 20 days incubation is again within 96% (0.379 L CH_4/g VS) of the 55 day methane yield. Therefore it can safely be suggested that for analysing a solid waste sample in the tube, the analysis can be completed within 6 to 20 days depending on the objective of the experiment. Instead of running an experiment for a long period of 2 months, a mere 3 weeks is sufficient to obtain a comparable yields and kinetics data.

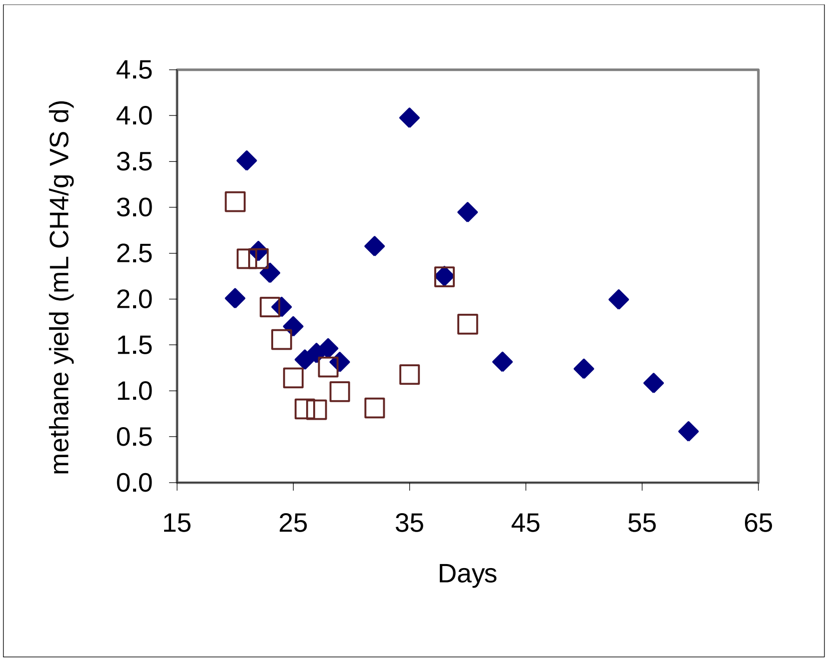


Figure 4.20 Daily gas production of the tubes corresponding to the ones marked ‘x’ in Figure 4.19, focusing from 20 to 59 days of incubation. The clear square is equivalent to the clear x while the black diamond is equivalent to the bold x

4.3.5.2 Mixing

On both occasions that the 18.8 OLR food waste tubes were mixed hourly versus mixed once daily, the methane yield was restricted. In Test 1, methane yield after 20 days incubation was 22.5% lower under frequent mixing producing just 0.347 L CH_4/g VS compared to 0.448 L CH_4/g VS with less mixing (refer Figure 4.21). The repeatability of Test 2 further confirms the finding that less mixing increases methane production. A maximum of 0.499 L CH_4/g VS was generated when tubes are mixed once daily but dropped by 21.7% once mixing frequency is heightened to hourly.

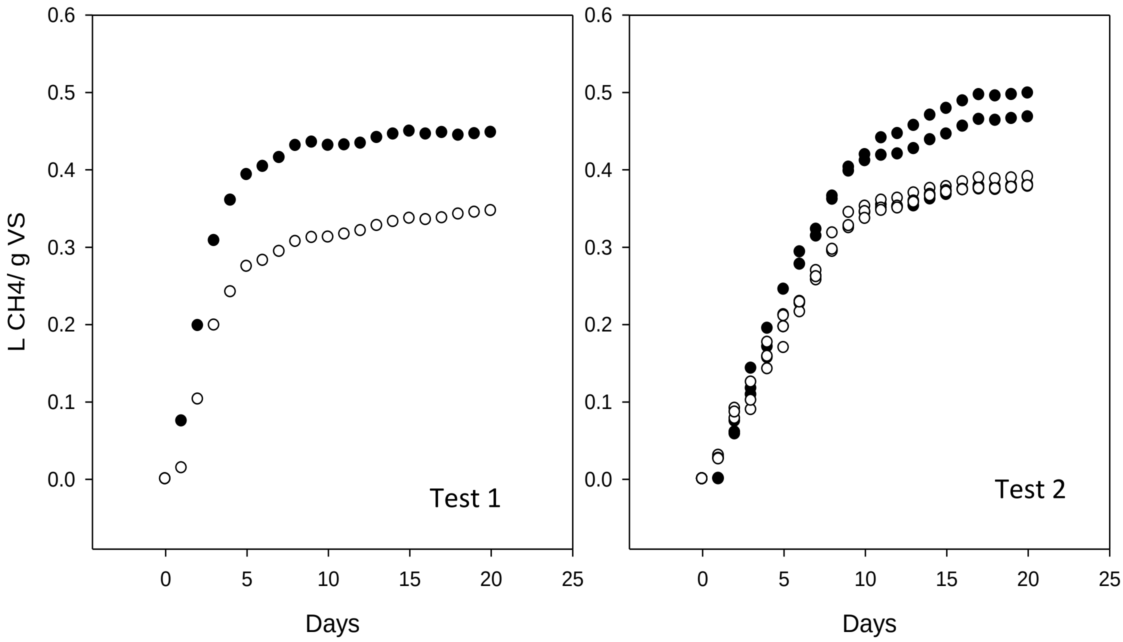


Figure 4.21 The effect of mixing hourly compared to once a day mixing to methane yield. The test was repeated twice, Test 1 and Test 2 (both at 18.8 g VS/L). The black circles represent daily mixing while the white circle represents hourly mixing

A later study seemed to suggest the insignificance of mixing frequency to the methane production. This is because, when 18.8 OLR food waste was digested and mixed once a day and once a week, both high and low methane yield was observed (Figure 4.22 (a)). The once a week mixing produced 0.34 and 0.1 L CH_4/g VS while daily frequency produced 0.32 L and 0.06 L CH_4/g VS. Eliminating mixing did not improve the observation. Instead Figure 4.22 (b) further reinforces the notion that mixing can be done either once a day, lesser or skipped without seriously affecting the test precision.

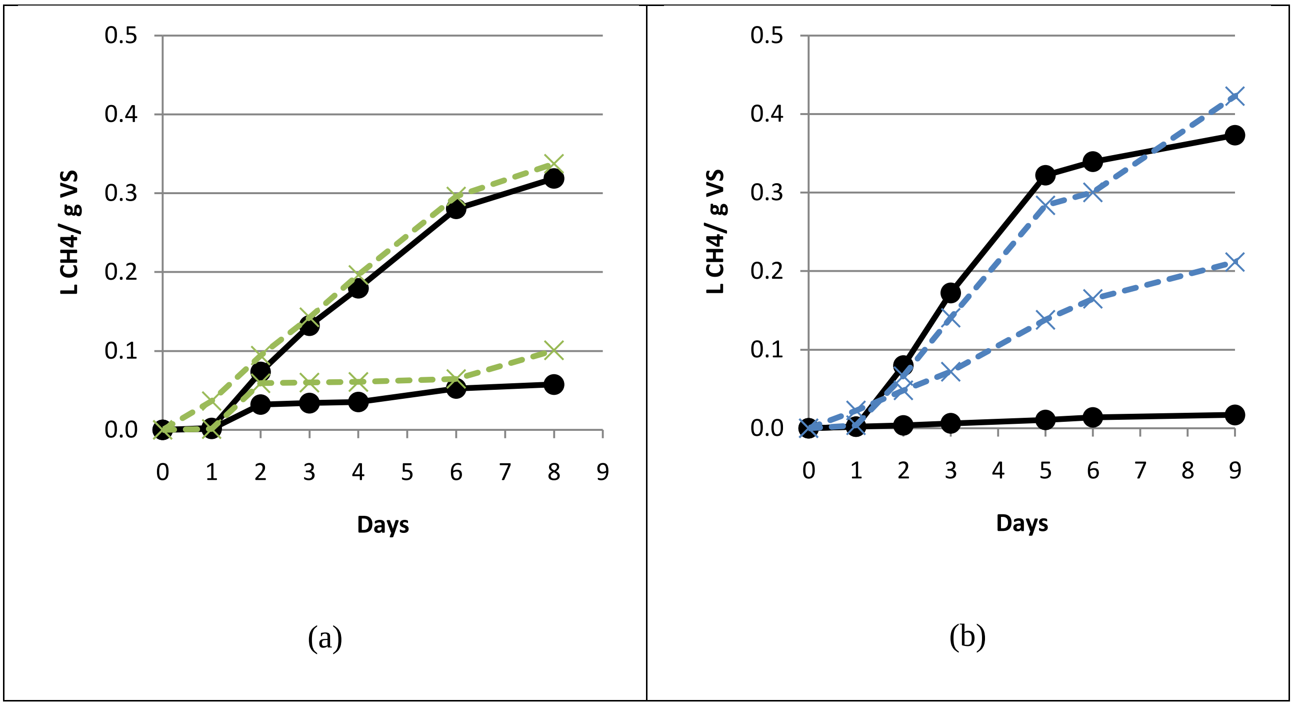


Figure 4.22 The effect of reactor mixing on the methane yield of food waste when (a) mixed weekly and daily at 18.8 OLR and (b) mixed daily and not mixed (OLR of 14.4 g VS/L. d) throughout the incubation period. The methane yield from mixing weekly and unmixed is represented by the x with broken lines (green for weekly and blue for unmixed). The black circles with solid line represent reactors that are mixed daily

4.3.5.3 Nutrient supplementation

As demonstrated by Figure 4.23, no clear significant advantage arose from supplementing the tubes with nutrients when digesting 25.1 g VS/L. d of food waste. The methane yield after 20 days with extra nutrients had an average of 0.38 ($\pm$ 0.04) L CH_4/g VS. The tubes without added nutrients had one replicate which produced a maximum methane yield of 0.37 L CH_4/g VS. These two values were not that far from each other and hence nutrient supplementation seemed unnecessary when analysing for food waste methane potential.

A repeated test where 60 ml trace element was added to 18.8 OLR food waste further supports this notion and exhibited a similar varied response to the nutrients addition in the batch test (refer Figure 4.24). One of the triplicates with nutrient supplementation yielded higher methane compared to the tubes without supplementation, reporting an increase in

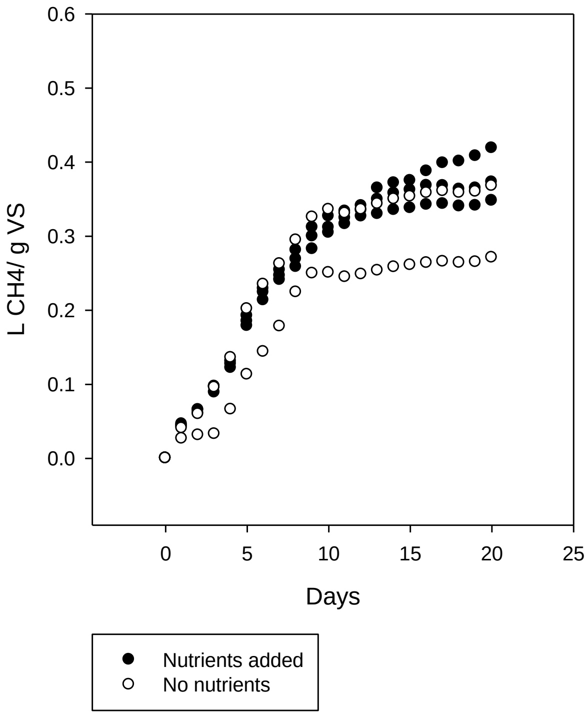


Figure 4.23 The effect of nutrient addition on methane potential of food waste. The black circle indicates nutrients are added while the white circle without any supplementation

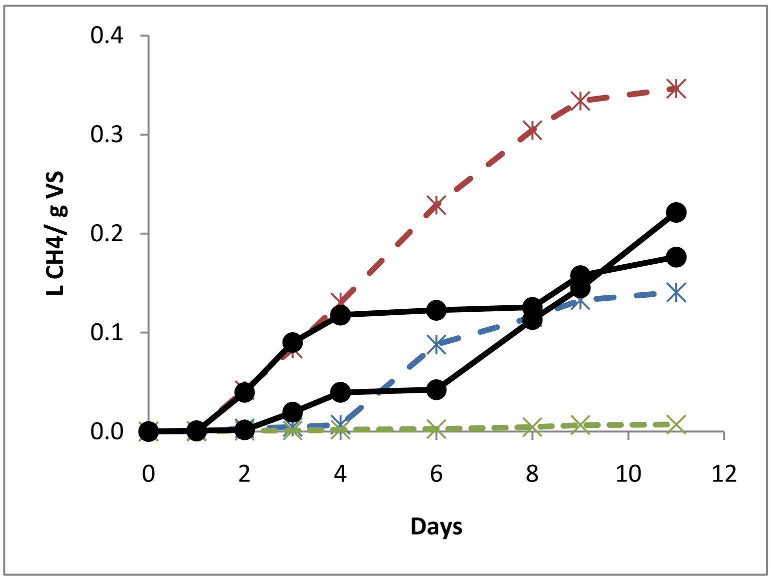


Figure 4.24 The response of 18.8 OLR food waste methane potential to the addition of nutrients. The black circle indicates nutrients are excluded while the dotted lines signifies nutrients are added to the tube test

methane yield by 37%. However, at the same time, two of the triplicates with added nutrients produced a methane lower than when nutrients was excluded. The former reported a maximum yield of 0.22 L CH_4/g VS compared to a maximum of 0.14 L CH_4/g VS with the latter. None of the nutrient supplemented reactors soured, as evidenced by the neutral pH at the end of incubation of around 7.22. It seems, a representative methane potential value for the food waste can be achieved without having to add extra nutrients.

4.3.5.4 Reactor headspace

The 18.8 OLR is equivalent to 120 g of food waste (wet weight). By maximising the 3.4 L reactor headspace, the food waste and seed amount was doubled to 240 g and 3 L, respectively, but still maintaining an OLR of 18.8 g VS/L.d. Instead of a 56% void reactor headspace, the increase in substrate and seed loading left only 12% of empty reactor headspace. As shown by Figure 4.25, the reduction in reactor headspace had no significant effect on the test yield or rate. The highest methane yield was obtained at 0.39 L CH_4/g VS for one of the 56% void headspace duplicate. However, the other pair of the 56% void headspace duplicate achieved methane yield quite similar to ones incubated at 12% void reactor headspace. The former produced 0.26 L CH_4/g VS while the latter had a maximum of 0.29 L CH_4/g VS out of the three replicates.

Despite the reduced headspace having no affects on substrate biomethanization, it seems however, as depicted in Figure 4.25, an improvement over replicate reproducibility was seen. When only 12% void headspace was provided, the methane yield between the three replicates was closer, having a standard deviation of 0.04 for an average methane yield of 0.26 L CH4/g VS. On the other hand, when a larger headspace of 56% was provided, the standard deviation more than doubled to 0.09 for an average yield of 0.33 L CH4/g VS. The results here suggest the influence of reactor headspace over replicate variability in a test run.

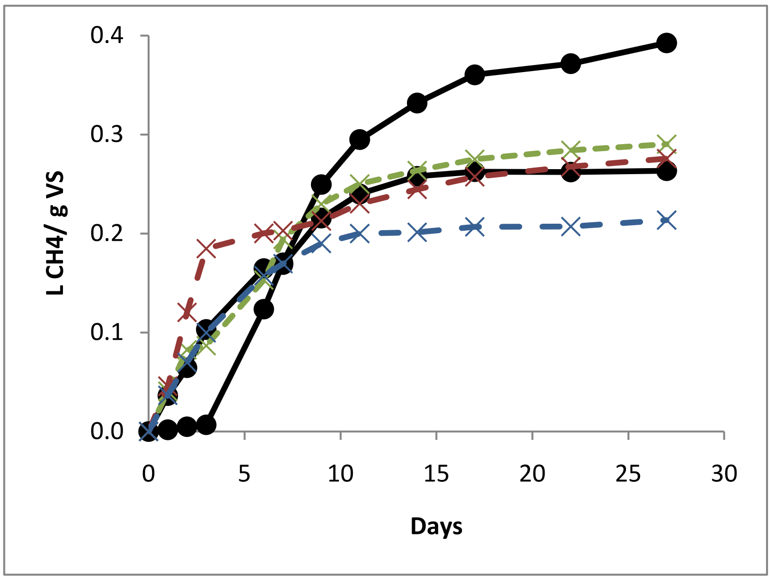


Figure 4.25 The effect of void reactor headspace on methane potential test of 18.8 OLR food waste. The bold circle represents a 56% void reactor headspace while the x with dotted lines indicates a 12% void reactor headspace

4.4 DISCUSSION

4.4.1 Tube Design

Unlike glass which is prone to cracking, breakage or even explosion, PVC is more durable and light-weight. Furthermore, the totally enclosed pipe provides an internal dark environment suitable for microbial growth, discounting the need of foil-wrapping the reactors. The tube design has been proven to accommodate a relatively large sample of 120 g (w/w) food waste. As verified in Section 4.3.5, there is possibility to further increase the sample size to 240 g (w/w) considering the tube volume is 3.4 L.

4.4.2 Seed Quality

Testing of a large sample size can be made possible provided enough seed volume is present. In this study, a ratio of substrate to seed ratio (by volume, v/v) of 1:12.5 or 8% sample content was found suitable for a healthy anaerobic process of food waste in a batch system. A substrate to seed ratio lower than 1:29 (v/v) has been reported by Forster-Carneiro et al. (2008), Neves et al., (2004) and Raposo et al. (2006) to contribute to a high maximum specific production per load. The higher seed volume added meant a reduced substrate or solids sample. Angelidaki et al. (2006) when testing various substrates to seed ratio by varying the substrate concentration (1.5-30% TS) found the methane yield of 0.41 L CH_4/g VS was obtained at 1.5% and 3% TS. However, only 68-83% of this value was achieved at 4.5-9% TS. The high methane yield at 1.5% TS was attributed to complete anaerobic biodegradation of the organic fraction of municipal solid waste (OFMSW), achieved in those assays with lower substrate to seed ratio without volatile fatty acid (VFA) accumulation reaching inhibiting levels (Angelidaki and Sanders, 2004; and Raposo et al., 2006).

In the case of Xu et al. (2002), a substrate to seed ratio of 1:0.5 led to a complete process failure. No methane was generated and pH dropped from 6.7 at start to 4.0 after 2 days which remained acidic thereafter. The continuous production of acetic acid and a lack of it being efficiently processed into methane is thought to have caused process failure (Raposo et al., 2006). Mathematical means for estimating seed volume have been derived but they require an assumption of substrate hydrolysis (Angelidaki and Sanders, 2004), which are not easily estimated. Clearly, for some substrates, lowering the substrate to seed ratio may be necessary for evaluation of the maximum rate of methane production (Chynoweth et al., 1993).

Seed provides an initial source of anaerobic bacteria to initiate anaerobic decomposition, in turn producing methane. As was shown earlier in Section 4.3.1, food waste contains a small amount of methanogen evidenced by the methane production during the first five days of incubation. However, it may take a couple of months for a strong bacteria population to develop and capable of decomposing the material and producing methane at a faster rate. Therefore, the quality of seed in an anaerobic digestion system is an important factor to be considered to dramatically improve the test time. A viable seed can be derived from the environments in which the anaerobic methanogenic decomposition of organic compounds occurs naturally, for example, anaerobic sewage digesters, anaerobic lake sediments or from animal faeces.

This study has seen no significant effect of the origin of the seed on the food waste methane yield. The assumption that acclimatised seed will eliminate the need for extended incubation period as well as exhibiting better decomposition rates compared to a sludge seed, was not proven true in this research. In fact, in our case, the seed grown in the laboratory for 190 days fed on similar substrate (food waste) gave a methane yield lower than a DSS seed, as demonstrated by Figure 4.8.

Vedrenne et al. (2008) also obtained a similar result when assaying animal manure using three different seed sources; laboratory grown seed, sludge of WWTP and digestate off winery waste reactor. The difference in the maximum methane yield between acclimated seed sourced from the laboratory and the one sourced from the WWTP varied by just 0.012 L CH_4/g VS. However, the time taken to reach 90% of the maximum methane yield was significantly reduced (up to 13 days) with the use of seed from WWTP compared to that from a laboratory reactor. Vedrenne et al. (2008) suggested these results are due to the proportion of active biomass and the diversity of this biomass (hydrolytic biomass versus methanogenic) contained in each inocula.

Unless the batch tests are done frequently and on an ongoing basis, it may prove impractical to maintain a reactor requiring regular feeding and wasting. Aside from the time consuming factor involved in running the continuous reactor, there are also cost issues with sourcing, transporting and storing feedstock as well as any digester heating and mixing requirements. Hence, it can be surmised that unless seed cannot be sourced easily and quickly from a local plant the growing of seed in a laboratory should be avoided considering no special advantage arises from its use.

The volume of seed added is an important factor since, as discussed earlier; a restricted seed volume causes process overloading. However, in a batch assay test, a low seed volume where the production of methane from the seed be lower than 20% of the total methane production (seed + substrate) (Vedrenne et al., 2008; and Hansen et al., 2004) has been suggested. This is to ensure minimal biogas production from the seed which may blur the contribution from the substrate. This research recorded less than 13% methane contributed from seed blank compared to the total methane production. If the production of the seed is higher, further incubation with a lower seed to substrate ratio is advised.

At the same time, a low seed volume also increases the allowable size of the substrate sample. In an attempt to improve the seed necessary for the purpose of the new design, this research looked at utilising a thickened seed. The hypothesis is that by increasing the total solids content of the seed, the seed microbes can be concentrated, therefore less seed volume is required for the same amount of substrate loading.

Unfortunately, as shown by Figure 4.9, despite the increased total solids (by 2.4 times) with the concentrate (settled layer), the percentage of methane production was similar to that of the original DSS. No significant advantage was achieved by gravity settling the seed sludge prior to its use. Published reports of similar investigation were mainly concerning manure slurry as the test subject. An increase of total solids in the settled layer by 2.7 times was recorded by Kaparaju and Angelidaki (2008) with digested manure. Instead, Ong et al. (2000a) found the settled layer barely thickened (~7.4 % TS contents for bottom and original slurry) after passive settling. A more significant improvement was seen for the top layer (12.8% TS) producing the most methane (400 L) and biomethanation rate. This difference was credited to the higher amounts of degradable matter such as carbohydrate and cellulose in the top layer compared to the proteinaceous materials and lignin with the settled layer. However, since Ong et al. (2000a) did not conduct a similar methane potential test using the slurry in its unaltered form, it is therefore inconclusive whether sludge thickening did actually help improve methane production in their study.

The methane potential of the separated fractions would also depend on how the fractionation was performed. Prolonged exposure to the air during the separation process may adversely affect the methanogens. The brush sieve and screw press method in the study by Kaparaju and Rintala (2008) was inefficient to recover the biogas potential from the fibres as most of the organic material was lost in the liquids that were separated. Since segregation of the seed sludge into various fractions does not appear to help in obtaining a fraction which could produce methane from the test substrate at a reduced seed volume, this research continued with using DSS as is collected at the WWTP.

In ISO 11734 (1995), pre-digestion of sludge is recommended when preparing seed for an anaerobic assay test. This research suggests a 5-7 days pre-digestion period without mixing, feeding or any supplementation and stored without oxygen at mesophilic temperature. Pre-digesting the seed between 5 to 7 days reduced the background gas production which could otherwise affect the test precision. However, carrying pre-digestion longer, for example 14 days, is not advised as this can impede methane production by prolonging the onset of methane production by the anaerobic bacteria.

In this research, the DSS sourced from the Christchurch Wastewater Treatment Plant was operated at a mesophilic temperature of 38°C. For use in the batch tests, the seed was first adapted to the test temperature of 35°C, by storing it in a temperature controlled room in the lab at 35°C for seven days. This finding is in line with several researchers who have seen benefit from pre-digestion of seed prior to application. Birch et al. (1989) found that by predigesting the seed for 5 days the background gas production was reduced. No unacceptable increase in either the lag or incubation period was observed in response. More recently, Hansen et al. (2004) held seed from a thermophilic biogas plant to 55°C for three days, Maya-Altamira, et al., (2008) for approximately 1 week at 35°C and 2 weeks at 35°C by Moller et al., (2004). These researchers believed pre-digestion helped remove most of the remaining methane production and readapted the inoculum to the test temperature. Methane production rates and the final yields were generally higher at temperature closer to that of original seed (Angelidaki et al., 2006).

4.4.3 Substrate Loading

A 120 g food waste in 1.5 L working volume is equivalent to 18.8 g VS/L organic loading rate (OLR). As shown by Figure 4.7 and Table 4.3, the OLR can be maximised to 25.1 g VS/L. d. without souring. However, the methane yield declined by 26% from a maximum methane yield of 0.499 L/g VS for 18.8 g VS/L. d. after 20 days.

Results from this study especially concerning Figures 4.8, 4.13, 4.16 and 4.17 indicates that the loading rate of 18.8 g VS/L. d., suitable for testing food waste in the tubes, is not applicable to other solid substrates. Sucrose and starch can only reach 6 g VS/L. d OLR, half of that for rice. The measured methane yield decreased from food waste to rice, starch and followed by sucrose. The measure of methane yield gives an indication of the substrate biodegradability. For a slowly degradable substrate a higher OLR is suggested, whereas for an easily degradable substrate a small OLR is recommended instead.

When a substrate is loaded in excess, a considerable fraction of chemical oxygen demand (COD) as VFA remained in the liquid phase, suggesting an inhibition of the methanogenic process that was likely due to the accumulation of long chain fatty acids (Neves et al., 2008). This is especially so for wastes with a high lipids content where a slower hydrolysis rate can be induced. The most efficient methane production rate and a low accumulation of VFA were reported for waste with excess soluble carbohydrate, signifying a higher acceptable OLR.

Lay et al. (1997) have shown that the methane production potential of organic wastes depend on their chemical nature. For example, the volume of methane (in L) from each gram VS varied between sludge cake (0.450), meat (0.424), carrot (0.269), rice (0.214), potato (0.203) and cabbage (0.096) even when the seed quantity and sample size was similar. These wastes represent different chemical components of waste including (i) proteins and lipids for meat and sludge cake, (ii) starch for carrot, rice and potato and (iii) cellulose for cabbage.

Cellulose is readily degradable but becomes less degradable or even refractory when incorporated in a lignocellulosic complex (Neves et al., 2006). Similar to our finding, Ueno et al. (2001) also observed a stable methane production on both glucose and cellulose at relatively low dilution rate but cellulose degradation rate declined with increasing dilution rate. Cellulose hydrolysis is thought to be the rate-limiting step in cellulose digestion. Ueno et al. (2001) suggested that a microbial population shift may be influencing the difference in methane yield between various substrates and various dilution rates. Microbial population shift is defined as a change of microbial population where different substrate or carbon sources lead to the predominance of different microorganisms from the same seed habitat.

4.4.4 Biodegradability of Food Waste

The prepared food waste (on % VS basis) consisted of 17.9% protein, 12.6% fat and 69.5 % total carbohydrate (carbohydrate+NDF). Based on this composition, the elemental formula is estimated to be $C_{5.73}H_{9.37}O_{3.67}$. This is in agreement with the collected household waste in the study by Lokshina et al. (2003) having 19.5% protein, 9.9% lipids and 70.6% carbohydrate. A similar molecular formula of $C_5H_9O_3N$ was employed by Nopharatana et al. (2007) when analysing methane potential of municipal solid waste. Based on this elemental and chemical composition, the theoretical methane potential of the food waste sample in our research is calculated to be 0.50 L CH_4/g VS. Similar value between 0.40 – 0.50 L CH_4/g VS has been reported by other researchers when analysing methane yield of organic fraction of municipal solid waste, organic household and restaurant waste (Forster-Carneiro et al., 2008; Hansen et al., 2004; and Neves et al., 2008).

The above maximum food waste methane yield per g VS added was obtained at an OLR of 18.8 g VS/L. As shown in Figure 4.8, the methane yield of the tube reactor increases with an increase in loading rates. This is the case until the OLR reaches a certain threshold which is 18.8 g VS/L. This is not the OLR where methane production is maximum, but is the highest OLR the process can tolerate before overloading occurs, methane production steeply declines and pH falls. It seems, the methane production of food waste was limited under two conditions; (i) at the lower loading rates of less than 14.1 g VS/L, and (ii) at a higher loading rates more than 18.8 g VS/L. At lower OLR, the low substrate appears to be the limiting factor as restricted carbon source is available for anaerobic microorganisms to grow. On the other hand, at the higher OLR, the substrate provided may be excessive for the slow growing methanogenic bacteria, hence overloading occurs. In this situation, methanogen could be the limiting factor as evidenced by the acidic pH of 5.5.

That higher yields can be achieved using an optimum OLR has been experienced by many researchers. In the study by Toorkian et al. (2003), the methane production capacity of slaughterhouse effluent increased steadily up to an OLR of 27 kg SCOD m^{-3} d^{-1} with the maximum methane production of 283 l kg^{-1} VSS d^{-1} obtained at an OLR of 17.4 kg SCOD m^{-3} d^{-1}. From then on, the methane production declined and eventually decreased to 199 L CH_4 kg^{-1} VSS d^{-1} when OLR was 39.5 kg SCOD m^{-3} d^{-1}. Starting at OLR of 4.42 g VS/L. d., Fernandez et al. (2005), found the highest OLR the semi-continuous reactor can accept for digesting OFMSW was 5.07 g VS/L. d. generating 0.43 m^3 CH4 kg COD^{-1} d^{-1}. The methane yield dropped to 0.34 and eventually 0.30 CH_4 kg COD^{-1} d^{-1} when the OLR rose to 5.92 and 7.50 g VS/L. d., respectively.

At OLR below the optimal (at which VFA concentration is the highest), VFA concentration decreases as a consequence of less metabolization by the methanogenic bacteria (Mtz-Viturtia et al., 1995). At low loadings, the utilisation of substrate in the batch reactor was limited by the amount of substrate and not by the amount of inoculum. As observed in this research, the pH for the low loadings was around 7.4 which indicate the process is healthy and has not soured. However, the methane yield at these low loadings (between 4.7 to 14.1 g VS/L. d) did not reach its full potential but instead was constantly lower than 0.35 L CH_4/g VS. This is also evident in the work by Nopharatana et al. (2007) as the methane production rate more or less doubled from 36.1 to 80.3 L CH_4/d in response to the substrate increasing from 4.3 kg MSW (dry weight) to 7.5 kg.

Mtz-Viturtia et al. (1995) found that at higher loads, the overall VFA concentration decreases, and the same happens at lower loads. It seems that, by increasing the OLR, hydrolytic step efficiency declines. Suppression of methanogenesis by the high VFAs concentration at an initial food waste loading of 10 g VS/L. d. in the study by Vavilin et al. (2004) seems to escalate to total inhibition of methanogenesis and hydrolysis at very high VFA when the loading was increased to 50 g VS/L. d.. A drop in pH to 4.0 corresponded to VFAs accumulation, signifying the methanogenic population becomes insufficient to prevent VFAs accumulation causing the imbalance between processes of hydrolysis/acidogenesis and methanogenesis (Vavilin et al., 2004). A rather high percentage of microbial seeding is recommended to counteract this effect especially at very high food waste loading. Figure 4.26 is a sketch showing how OLR affects the CH_4 production and VFA progression, in general terms.

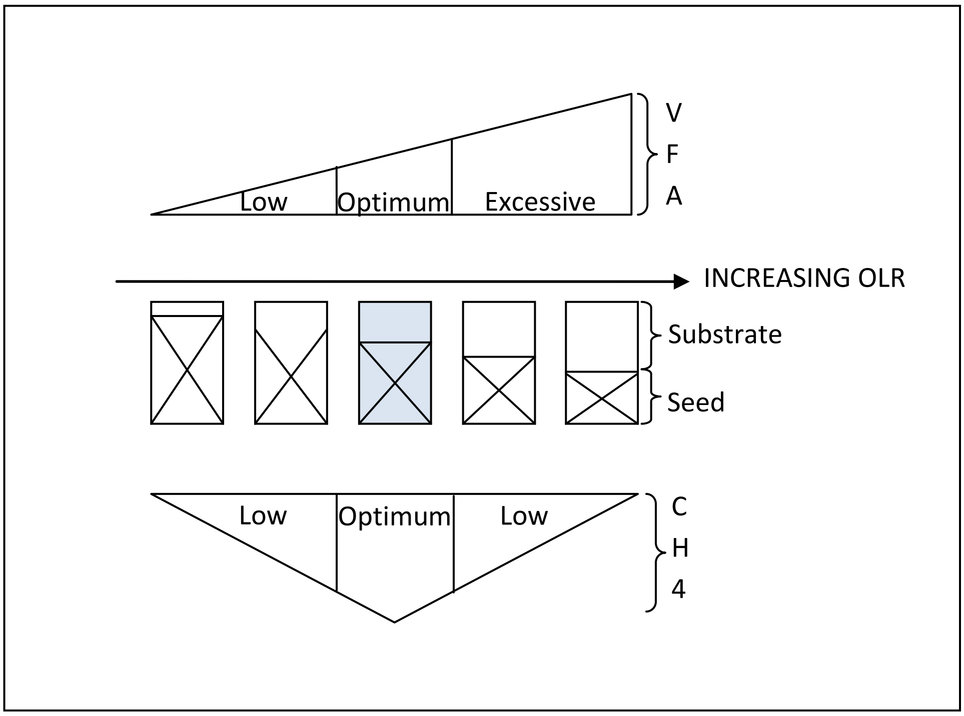


Figure 4.26 The relationship between the reactor OLR to the VFA production and uptake as well as the methane production

4.4.5 Tube operating procedure

4.4.5.1 Mixing

The same argument as above may apply when the tube is frequently mixed. Our finding shows that the methane yield is roughly 20% lower for an hourly mixed tube compared to when it is only mixed once daily. The effect is more pronounced under high substrate to seed ratio in batch systems where methanogenesis is inhibited, this is because the produced VFA was distributed homogenously in the bottle when vigorously mixed (Kaparaju et al., 2008).

Excessive VFA diffusion into the initial areas of methanogenesis, where concentration of microorganisms is low, suppresses microorganism growth. Thus, the negative effect of frequent mixing may be interpreted as a significant reduction of a number of possible initiation methanogenic centers (Vavilin et al., 2004). On the other hand, under minimal mixing the most viable initial areas could survive and expand over the reactor volume becoming the initiation of new methanogenic centers. The concept of methanogenic centers by Vavilin et al. (2004) considers waste and inoculums are randomly distributed over the reactor space, and that only part of the existing initial methanogenic areas can survive and expand in space during digestion.

The positive effect of minimal mixing could also be ascribed to a better syntrophic association between H_2 producing and consuming organisms, as observed by Kaparaju et al. (2008) using Fluorescence in situ hybridisation (FISH). Minimal mixing where reactors are thoroughly shaken by hand for about 1 minute twice a week was found to improve methane production by 12.5% compared to continuous mixing. Other researchers have also implemented minimal mixing when conducting laboratory experiments. Gungor-Demirci and Demirer (2004) mixed the 250 ml batch reactors manually once a day. O’Sullivan et al. (2005) stirred the 2 L batch reactors only at sampling times (usually daily), while the reactor was settled at all other times.

4.4.5.2 Experimental time

To obtain yields of solid organic decomposition in the tube at mesophilic condition, a 20 day incubation period is sufficient as 95% of the maximum methane yield is already achieved. Likewise, a shorter period of only 6 days may be implemented for the purpose of preliminary or simple tests like establishing suitable OLR. Methane yield at 6 days generally represents 75% of the maximum yield. As no second peak was observed in the methane production curve over time, for easily degradable substrate like rice, food waste, starch and glucose, the common incubation period up to 60 days may not be necessary. Instead, long incubation period is more recommended for a slowly degradable substrate like cellulose. This follows the guideline by ISO 11734 that cite although an incubation period of 60 days is recommended, the test can be terminated sooner if > 50% of the theoretical value is reached, indicating a sufficient degree of biodegradation. With food waste, this research found it took an average four days incubation to reach 50% degradation when analysed in the tubes.

Nopharatana et al. (2007) also found a 20 day incubation period sufficient to achieve 95% value of the maximum methane potential for municipal solid waste. The quick degradation in their batch system may be due to the reactor contents consisting of acclimated seed, shredded substrate along with nutrients, buffer and trace elements supplementation. Neves et al. (2008) when conducting batch mesophilic digestion of restaurant waste used 85% of the theoretical methane potential as the measure of degree of biodegradation. The first assay to achieve 85% methanation was the one containing an excess of protein (after 23 days), followed by the assay containing an excess of cellulose (after 24 days), and excess of carbohydrates (in 30 days) and restaurant waste (in 32 days). Earlier, Neves et al. (2004) also used a 25 days incubation period as their biodegradability term since the maximum plateau in the production curves of kitchen waste biomethanation had been reached.

The research by Moller et al. (2004) found that the methane yield of straw after 60 days was 0.16 L CH_4/g VS, while after 110 days , the same substrate had a methane yield of 0.195 L CH_4/g VS (Moller et al., 2004). The graph of methane yield over time on pre-treated slaughterhouse effluent by Maya-Altamira et al. (2008) did not exhibit much difference in the methane yield obtained after 60 days and the final reading at 90 days. This small and non-significant effect indicates that extending the batch digestion further than 60 days is impractical. A sufficient incubation period of about 20-60 days has been shown to capture the representative anaerobic degradation and methane potential of the sample under examination.

4.4.5.3 Nutrients

In a batch anaerobic test like the BMP test, the original nutrient media recipe as suggested by Owen et al., (1979) contained extensive listings of vitamins, micro- and macro-nutrients. The nutrient media are sometimes modified, for example excluding vitamins when assaying maize in the experiment by Raposo et al. (2006). Some of the common of trace elements used by researchers in their batch tests includes iron(ii) chloride tetrahydride, boric acid, zinc chloride, copper sulfate pentahydride, manganese(ii) chloride tetrahydride, magnesium chloride hexahydride, aluminium chloride hexahydride and cobalt(ii) chloride hexahydride among others, (Raposo et al., 2006) and Zhang et al. (2003).

This research found that methane yield of food waste in batch tests using DSS at high levels can be achieved close to the theoretical value without having to add nutrients or trace elements. In fact, when added, no significant production over non supplemented reactors were observed. For both times the test was executed, the reactors without supplementation had replicates there were producing methane at par with one of the duplicates with added nutrients. Lokshina et al. (2003) and Forster-Carneiro et al. (2008) among others also excluded nutrients addition in the batch assays when determining methane potential of samples like slaughterhouse waste, household waste and OFMSW, without any detrimental effects.

The C/N ratio of 20 for the food waste sample in this research may have provided enough nutrients for the microorganism growth. A lower C/N ratio of 18 (Charles et al., 2009), 16.2 (Heo et al., 2004), and 14.8 (Zhang et al., 2007) for food waste has been shown to avoid ammonia toxicity issues and produce a healthy methane yield. Food waste samples collected from restaurants, food markets and commercial sources have been shown to contain a balanced micro and macronutrients (refer Table 4.9), hence the avoidance of extra nutrient is acceptable (Zhang et al., 2003).

Table 4.9 Elemental composition of food waste in the study by Zhang et al. (2003)

Components	Unit	Average value (standard deviation)
Total solids (TS)	% (w.b)	30.90 (0.007)
Volatile solids (VS)	% (w.b)	26.35 (0.14)
Fixed solids (FS)	% (w.b)	4.54 (0.21)
VS/TS	%	85.30 (0.65)
C (Total)	% (d.b)	46.78 (1.15)
N (Total)	% (d.b)	3.16 (0.22)
P (total)	% (d.b)	0.52 (0.08)
K	% (d.b)	0.90 (0.11)
Ca (Total)	% (d.b)	2.16 (0.29)
Mg (Total)	% (d.b)	0.14 (0.01)
S (Total)	ppm*	2508 (87)
NH_4-N	ppm	973 (571)
NO_3-N	ppm	118 (80)
Al	ppm	1202 (396)
Fe (Total)	ppm	766 (402)
B (Total)	ppm	12 (1)
Zn (Total)	ppm	76 (22)
Mn (Total)	ppm	60 (30)
Cu (Total)	ppm	31 (1)
C4	ppm	< 1
Cr	ppm	3 (1)
Pb	ppm	4 (3)
Ni	ppm	2 (1)

* Note: Based on wet base

The inoculums, if provided in sufficient amount, may also make the dependency on nutrient addition to be redundant. Maya-Altamira et al. (2008) established that sludge taken from the local WWTP for reactor seeding shows typical ratios of COD/TKN at around 10 and COD/VS at around 1.2 which provided enough nutrients for the assays as shown by COD/TKN ratio. The DSS used in this research is within this value with a COD/TKN ratio of 16. With nutrient supplementation in a batch test, there would be costs involved and possible toxic effects if excessive amounts were added. The need for extra nutrients is probably better justified when nutrients limitation are suspected. This could be when a poor seed source is used, or the testing of substrate low in nutrients or could be due to the washout of micronutrients (more prone under continuous reactor system). Under these circumstances adding supplementary nutrients could help stimulate the growth of bacteria and build a robust population.

4.4.6 Reproducibility of results

Testing of 18.8 OLR food waste was repeated several times over the course of the experiment, and a variation in methane yield was observed. This is clearly shown by Figure 4.5(b), a compilation between a number of runs and Figure 4.17, which involves a number of replicates in the same run. The methane yield for 18.8 OLR food waste is sometimes reproducible but there are also times that it is not. When rice is tested instead at 6.8, 10.2 and 11 OLR in the tube, the same irreproducibility with the results were observed. This was not expected since rice is more homogenous in nature compared to food waste. In fact, rice was chosen as the test material since the particle size and composition is uniform throughout.

The variation in laboratory measurement between triplicates in a batch assay was also observed in the experiments by Vedrenne et al. (2008). The maximum methane yield varied from 0.244 to 0.343 L CH_4/g VS for fattening pig manure and from 0.260 to 0.334 L CH_4/g VS for sows. The repeatability of a similar sample taken at different times were 0.296 L CH_4/g VS and 0.244 L CH_4/g VS, indicating the methane yield may vary with time. Hansen et al. (2004) argues that the difference between the highest and lowest methane yield not exceeding 0.156 L CH_4/g VS indicates that the experimental data for determining the methane yield of cellulose is acceptable. Likewise, for two measurements taken at two different times, a yield ranging between 0.315 to 0.439 L CH_4/g VS in their study was also proven statistically viable. The work by Hansen et al. (2004) and Jensen et al. (2007) reassures that the variation in the methane yields obtained in this research is typical. In addition, it was also pointed out, that although much effort is taken at dispensing uniform seed and the use of homogenous sample like cellulose powder, the result could fluctuate regardless.

Figure 4.18 indicates that irreproducibility may not only be a function of the seed and the substrate. Although the seed contributes minimally in terms of methane production (10–13 %), the large seed volume used (1.5 L) may have somehow influenced the reproducibility of test results. The microorganism distribution and/or contact with the substrate as well as the quality of the seed may exhibit a larger affect on test reproducibility than can be known through the experiments conducted here.

The OLR at which the test is conducted may also be an influencing factor. A more erratic anaerobic process may take place when the OLR is closer to its maximum tolerance. This was seen for both rice and food waste which was tested at a loading >75% of the max OLR. Both substrates had a standard deviation of $\pm$ 0.11 for an average yield of 0.246 and 0.210 L CH_4/g VS, respectively. The gap between replicates' methane yield seems to shrink at a smaller OLR for example when OLR is 50% of the maximum tolerance. This is in line with the observation by Toorkian et al. (2003) where indication of erratic behaviour was observed at high organic loads when digesting slaughterhouse wastewater. The instability was manifested by the widening and varied response of methane production per unit of biomass and SCOD removal curves at high loads. Guendouz et al. (2007) saw VFA production and uptake levels varied between runs of the same reactor and between reactors; variable ammonia levels between 1200-2000 mg N-NH_4 to 2000 during the tests were thought to have caused this irreproducible VFA behaviour. Guendoz et al. (2007) highlights that the transitory accumulation of VFA during the batch tests may suggest that VFA uptake rate plays a crucial role in the whole degradation kinetics driving the reproducibility of the yields.

As found in Section 4.3.5.4, there also stood a better reproducibility between replicates if less void headspace is provided in the test vessels. This research saw an improvement of reproducibility by 50% when the void reactor headspace was reduced from 56% to 12%. This finding is in line with the suggestions by ISO 11734 (1995) and Pagga and Beimborn (1993) that a reactor headspace should be around 10% - 30%, partly because of the control a reactor headspace exhibits over test reproducibility. The reduced headspace means a larger sample size can be accommodated, resulting in a higher gas production. It is the assumption of this research that, the restricted space within the reactor and the corresponding overpressure, allowed the produced biogas to flow more into the external container rather than being retained within the reactor itself. The efficient capture or collection of the produced gas from the reduced void reactor headspace may have led to a more precise gas quantification which in turn drives the improvement over test reproducibility.

To ensure that a representative result is being reported especially in the case where irregularity is an issue, the following are suggested as most efficient ways to work around it:

i) Avoid testing a substrate at an OLR above 75% of the maximum. Instead, testing is recommended at an OLR of 50% as a higher reproducibility can be anticipated. The maximum OLR is determined after trying different substrate loadings and the highest loading without reaching an acidic pH (< 6.5).

ii) In the case where the result between replicates is widely varied, it is recommended that the average value be reported. For averaging purposes, consider the replicates with less variability, preferably where standard deviation is less than 20%.

It is important to include a duplicate seed blank (the least) in each run to check whether seed could be one of the irregularity contributors for that run.

Hansen et al. (2004) note that excessive variation between replicates in the same run occur because the distribution of the inocula in the reactors is not sufficiently homogenous. On the other hand when a large variation on the same sample was seen between different runs, it may suggest that the seed was inhibited or of other reasons like lack of micronutrients or excessive presence of unsuitable compounds like lipids, produced too little methane (Hansen et al., 2004).

4.5 CONCLUSION

One of the aims of this research is to develop a tool to be used as a batch test to measure the anaerobic decomposition of solid organic sample. This tool (dubbed tube) would be able to cater for a large range of samples and its most significant feature is the wide opening (about 10 cm) that would enable sample analysis ‘as is’ and in large volumes. Loading of food waste up to 240 g wet weight has been proven viable. In addition, the design does not require bulky, expensive, high pressure vessels instead are set-up using cheap easily available pipe material and fittings.

For a batch system, as in this research, where the acid and methane producing phases are together, a balance between these processes is important. It is necessary to optimise the initial amount of the test substrate relative to the seed volume. The OLR is dictated by the substrate biodegradability; for a less degradable substrate, a higher OLR is permissible however a long incubation period more than 20 days is recommended to ensure complete degradation. Not only does the right OLR give a high maximum methane yield, it also ensures a healthy digestion process without approaching inhibitory levels.

The irreproducibility appears to have been a factor of the seed quality rather than the substrate homogeneity. As illustrated in Figure 4.14, although every care is taken to be consistent and repetitive in seed collection, storage and use, the seed seems to behave differently for some runs. There were also occasions that a large variation between seed blanks was observed in the same run. As some of these factors are beyond the researchers' control, the seed variation issue can be addressed by consistently using the same aged seed e.g 7 days and to do two seed blanks each time. The collected seed can be stored at incubation temperature for 4 to 7 days without any ill effects to anaerobic decomposition. The use of 7 day old DSS as seed seems to have two benefits: (i) a more stable substrate degradation, and (ii) a smaller amount of methane which would not blur the result of methane production from the test material.

It is crucial to prevent volatile fatty acids accumulation within the seed particles which may affect the adaptive methanogenic competence of the seed, especially so in a batch test. The use of a large inoculum appears to counteract the acidification allowing for a successful digestion without pH adjustment. Extra attention to details such as optimal growth conditions followed by careful assessment of the result are highly recommended.

Finally, it is concluded that the interrelationship between organic loading rate, substrate biodegradability and reproducibility of test result seems to boil down to careful management of the VFA production and consumption to methane. This can only be achieved if overloading of reactor is avoided and the right balance between the various microbial groups is struck.

4.6 FURTHER WORK

Further research may help explain the influence of the seed quality to the tube test results, especially regarding methods to improve reproducibility. The research by Neves et al. (2004) offers some insight into this possibility. Granular sludge from an Upflow Anaerobic Sludge Blanket (UASB) reactor treating brewery prevented acidification better than suspended sludge from a local municipal sludge digester, especially when the waste/inoculums ratio was between 0.5 and 2.3 g VS/g VS. A higher methane production was observed with granular sludge use, suggesting that the number of active bacteria involved in the acetogenic and methanogenic conversions was much higher in that structured sludge. Furthermore, the architecture and spatial arrangement of granular sludge may have protected the bacteria located in the inner core (mostly acetoclastic) from adverse environmental conditions prevailing in the bulk medium. The work by Neves et al. (2004) did not provide enough details to conclude that granular sludge gave more reproducible results than a suspended DSS sludge. Although finding a full scale UASB reactor may be a challenge here in Christchurch, it may suffice to operate a laboratory scale reactor for the purpose of checking the effectiveness of using a granular sludge as seed.

The investigation in this research regarding the effect of various layer of DSS on methane generation earlier only involved sludge thickening through gravity settling. As shown by Moller et al. (2004), different separation techniques contribute differently to the ultimate methane yield, with the fractions having a higher VS concentration producing more gas. Separation using evaporation gave the highest methane yield followed by centrifugation and finally chemical precipitation. Thus, the effect of using a thickened DSS when determining food waste methane potential in this research can be expanded more using other thickening methods as above. It would be interesting to see if the same effects are obtainable to confirm our finding or whether a positive outcome is available.

Finally, since it is understood that there may be variations within the test replicates, it is highly recommend for any researcher to have knowledge of the substrate prior to testing. The chemical and elemental composition of the substrate should be known so that the theoretical value of the substrate as well as its biodegradation behaviour can be estimated. This is to serve as a benchmark and precaution to ensure that the test method applied is reliable.

CHAPTER 5

START UP PROCEDURE AND OPERATION OF A CONTINUOUS REACTOR

5.1 INTRODUCTION

A study commenced in 2000 found that 90% of solid organic waste anaerobic treatment plants in Europe were of single-stage continuous reactor system (De Baere, 2000). In addition, 62% were performed at mesophilic temperature. However, despite the increase in anaerobic digestion technology in recent years, this does not compare to the surge in composting. This was because anaerobic digestion was more expensive and many municipalities chose less risk and less investment.

The characteristic for continuous reactors is feeding on a frequent basis. This is usually done once or twice a day, with the simultaneous removal of material which has already finished digesting. The biogas production for continuous processes is generally higher and more regular than for batch systems, which goes some way towards accounting for their popularity in commercial applications.

Considering the importance of continuous reactor systems in commercial and municipal applications versus the risks it carries, most investors turn to laboratory investigations first. Research at the laboratory level usually involves batch testing and later verified in a continuous system or straight to the latter. Both continuous and batch-feed techniques are useful at evaluating toxicity and substrate biodegradability, while the former illustrates a closer simulation of a full-scale anaerobic operation.

This aim of this chapter is to provide information regarding starting, operating and maintaining a continuous reactor digesting organic waste. The information collected here would provide specific and simple guidelines covering methods of operation, threshold values, as well as treatment options should reactor performance decline. It is the intention that first-time anaerobic operators would find this chapter easy to grasp, follow and implement, and help them ensure a successful bench-scale trial.

5.2 METHODOLOGY

5.2.1 Reactors

Over the course of nearly 1.5 years of experimental time, several substrates have been digested in a continuous reactor under mesophilic temperature (35 $\pm$ °C). The reactor design has been detailed in the Methodology Chapter. The following describes the operating conditions of these reactors (refer Table 5.1).

The operating conditions for the meat, meat and sawdust, FW2.6 and cabbage reactors were a result of trial-and-error. Because it was the first time the Environmental Engineering laboratory (at University of Canterbury) ever conducted anaerobic digestion experiments, the operations and initiation of these four reactors were more of a learning experience. Therefore, the irregular ratio of feedstock, the low OLRs, long HRTs and reduced feeding frequency reflects this. The aim of running these four reactors (meat, meat and sawdust, FW2.6 and cabbage) was mainly to try and operate a reactor for a long period of time without approaching instability. After grasping the technique of reactor start-up, operation and maintenance, five other reactors (FW7, FW6, FW5, FW3 and FW1.5) were initiated fed on food waste as substrate. An OLR of 5 – 7 g VS/L. d was tried (based on literature) to find the optimum OLR for a continuous digestion of food waste. The lower OLRs of 3 and 1.5 serves to support the yields and performance observed from the FW6 reactor. The corresponding OLRs were achieved by varying the food waste to water ratio in the 500 g wet weight daily feed. A HRT of 30 days was opted to allow sufficient substrate degradation, retainment of biomass and prevention of reactor washout.

Table 5.1 Description of the continuous reactors

Reactor label	Feedstock (by wet weight, g)	Experimental period	HRT (days)	OLR (g VS/L. d)	Feeding frequency
Meat & sawdust	90:110:300 (meat:sawdust:water)	22/01 – 15/04/2007 (82 days)	52	2.1	Every 2 day s
Meat	180 (meat)	31/01 – 18/06/2007 (137 days)	166	1.8	Every 2 days
FW2.6	500 (food waste)	17/05 – 03/12/2007 (200 days)	90	2.6	Every 3 days
Cabbage	500 (cabbage)	10/07 – 26/11/2007 (139 days)	84	0.7	Every 3 days
FW7	500:0 (food waste:water)	25/01 – 04/02/2008 (10 days) 12/02 – 22/02/2008 (10 days)	30	7.0	Daily
FW6	437.5:62.5 (food waste:water)	26/02 – 06/03/2008 (9 days)	30	6.0	Daily
FW5	375:125 (food waste:water)	25/01 – 04/02/2008 (10 days) 12/02 – 13/05/2008 (91 days)	30	5.0	Daily
FW3	250:250 (food waste:water)	25/01 – 07/02/2008 (13 days) 12/02 – 13/05/2008 (91 days)	30	3.0	Daily
FW1.5	125:375 (food waste:water)	27/03 – 13/05/2008 (47 days)	30	1.5	Daily

Note: OLR: organic loading rate, HRT: hydraulic retention time

Cabbage, dog roll (meat representative) and the items for the laboratory-prepared food waste (FW) was purchased at a local supermarket, Pak n Save. Table 5.2 describes the solids content of the various feedstocks. The cabbage and food waste were cut into small pieces in a food processor. Then, these cut feedstocks, along with the dog roll, were stored in a refrigerator at -20°C. For use, the feedstock was taken out and kept in a temperature controlled room at 35°C to thaw overnight. The feedstock was then mixed with the respective mixtures e.g. sawdust or water at the set ratios and mixed well prior to feeding.

Table 5.2 The solid contents of the feedstock

Feedstock	MC% wet sample	TS% wet sample	VS%/TS%
Meat & sawdust	84	16	80
Meat	65	35	86
Food waste	72	28	84
Cabbage	93	7	91

Note: MC: moisture content, TS: total solids, VS: volatile solids

To maintain the hydraulic retention time (HRT), the same amount of feed and effluents were exchanged in terms of weight. Reactor feeding and wasting was performed following mixing, with the latter conducted first. Mixing was done first to ensure the reactor contents are equally distributed and hopefully exchanged with the fresh feed. The results for observing, maintaining and stability of the various reactors are discussed. This will be done by examining the biogas and effluent parameters.

5.2.2 Parameters

5.2.2.1 Biogas composition

A gas analyzer as described in the Methodology Chapter was utilized for establishing the biogas composition. The gases measured in percentage include methane (CH_4), carbon dioxide (CO_2), oxygen (O_2) and balance (assumed nitrogen, N_2 and trace gases). Hydrogen (H_2) was also detectable but was given as low, medium or high. Unfortunately no information regarding the H_2 concentrations explaining the above ranges was reported in the GA2000Plus

Landfill Gas Analyzer General Operating Manual. However, the gas analyzer has been calibrated against H_2 concentrations measured via a HP 5890 Gas Chromatograph (GC). The result pertaining to this work is given in Chapter 4. It is found that for a hydrogen concentration between 10-30,550 ppm, the Gas Analyzer reported a low H_2 category.

5.2.2.2 Effluent analysis

The pH was analysed on raw effluent, whereas soluble COD (SCOD), ammonia (NH_3-N) and alkalinity requires effluent centrifugation (for 15 minutes at 4.3rpm) and filtered with glass microfibre filter (LabServ). pH was taken with an EDT RE357 Microprocessor pH meter (Made in England). SCOD and NH_3-N was determined using Hach test 'n tube vials and Hach Spectrophotometer using Method #435 and #343, respectively. For ammonia, the salicylate method (method #10031) with a range of 0.4 to 50.0 mg/L NH_3-N was applied. A green colour develops if ammonia is present at 655 nm. If the ammonia value is off-scale, samples will be diluted with distilled water and reanalysed. Alkalinity is done to pH 4.5 titrated with 0.2N sulphuric acid (H_2SO_4). For volatile fatty acid (VFA) analysis on a HP6980 Gas Chromatograph, further filtration to 0.22μm and dilution is necessary to prevent GC malfunction. The same method as discussed in the Methodology Chapter was used.

5.2.3 Start-up Procedure

This research also looked at different start-up strategies that could be advantageous to usher the reactors into steady state successfully. The pros and cons of each strategy will be discussed. The start-up procedures tested were as depicted in Table 5.3.

Table 5.3 Description of Start-up Trials

Start-up Trial	Feedstock	Seed volume (L)	Seed pre-digestion	Feedstock (kg)	Buffer	Other reactor effluent	Trace elements
1	Meat	15	No	0.18	nil	nil	nil
2	Food waste (2.6 OLR)	15	14 days	0.5	50 g	nil	nil
3	Cabbage	14	7 days	0.5	50 g until Day 9	100 ml until Day 18	nil
4	Food waste (7, 5 and 3 OLR)	15	7 days	0.5	Nil	nil	5 ml each feeding
5	Food waste (7, 6, 5, 3 and 1.5 OLR)	15	7 days	0.5	50 g	nil	5 ml each feeding

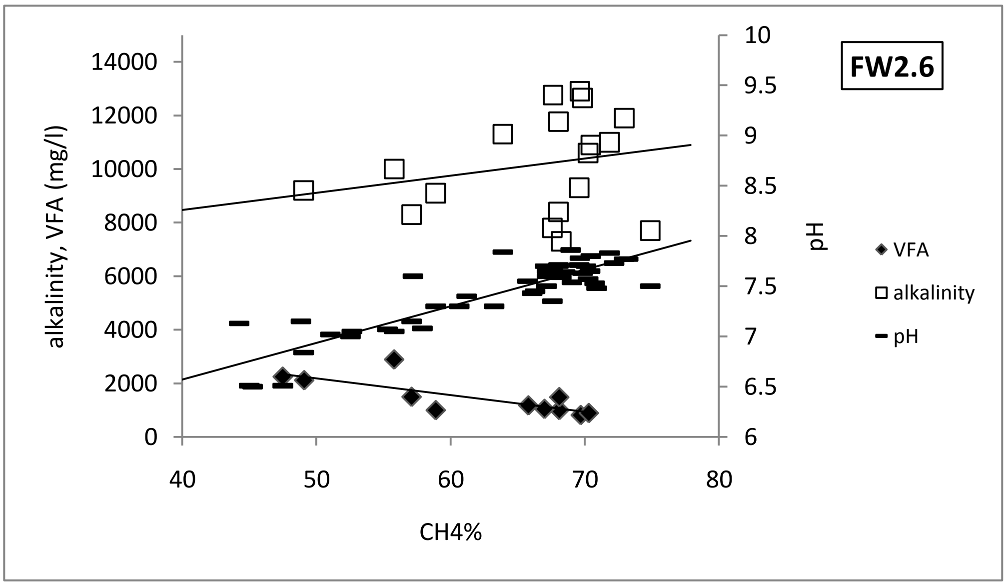
5.3 RESULTS

In this Section, results regarding a ‘healthy’ reactor will be presented, with examples from different substrates. Methods of starting up a reactor are also shown to convey the easiest, safest and quickest way of ushering a reactor into a steady state condition. Next, various indicators of reactor stability will be explored to identify its threshold values along with recommendation of a preferred indicator. By acknowledging and monitoring these parameters continuously, a reactor’s decline in activity can be detected, investigated, resolved and prevented.

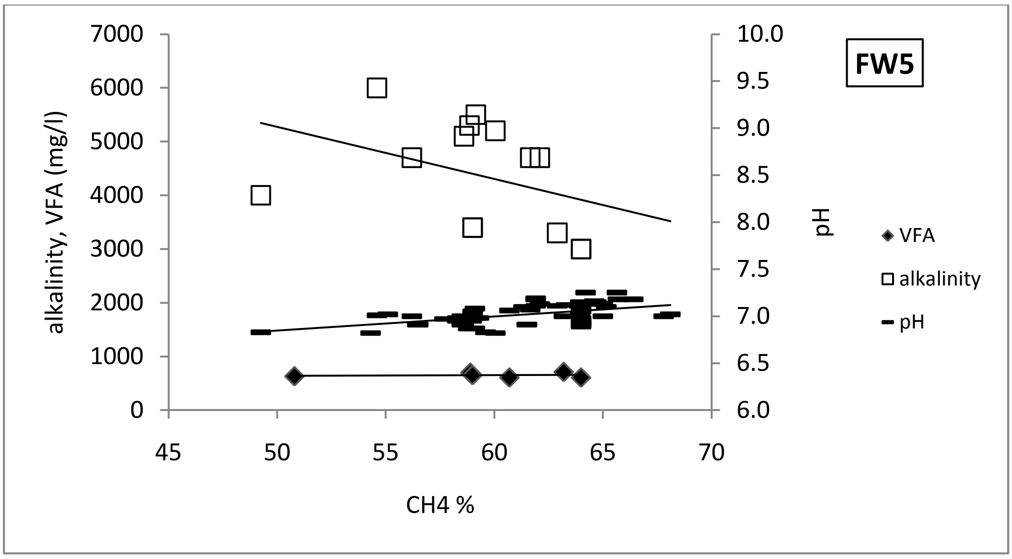
5.3.1 A stable reactor

Figure 5.1 is an example of two healthy reactors operating at steady state. Both reactors are fed on food waste but at different conditions. One was loaded with 2.6 g VS/L. d (labelled

FW2.6) while the other at 5 g VS/L. d. (labelled FW5), with 90 and 30 days hydraulic retention time (HRT), respectively. As can be seen, the methane content of both reactors at steady state ranged between 50 – 70%. The pH for these reactors were around neutral (pH 7.0) with the FW2.6 having an increasing pH trend along a higher CH_4%. At 50% CH_4, pH was around 6.8-7.2 while at 70% CH_4, the pH was higher at 7.5-7.9. This is not surprising since for FW2.6, the volatile fatty acids were highest (2300 mg/l) at the lowest methane percentage of 48%. Further decline in VFA concentration to 900 mg/l saw methane rising to 70%. On the other hand, for FW5, the pH was almost stable around 7.0 ($\pm$ 0.3) for the same methane readings. Again, the VFA concentration coincides with the pH and methane percentage behaviour where VFA was around 651 ($\pm$ 44) mg/l for CH_4 of 51% to 64%. The pH and VFA concentrations seem to suggest a suitable threshold value for a healthy anaerobic digestion process more than exhibiting a stressed or unhealthy condition. A large variation in the alkalinity data was seen across the 50 to 65 percent methane range, with the FW5 displaying a decreased alkalinity trend towards an increasing CH_4 percentage. The alkalinity was 4000 mg/l CaCO3 at 50% CH_4 and 3000 mg/l at 64% CH_4.



(a)



(b)

Figure 5.1 The conditions of healthy reactors looking at the VFA, alkalinity and pH of food waste digestion at (a) 2.6 OLR and (b) 5 OLR. All data after 10 days start-up

5.3.1.1 Substrate effects

When meat was digested, a lower methane percentage was observed at about 20 – 40% (Figure 5.2). As shown by Figure 5.3 the ammonia content of the meat digester is three times more (about 4500 mg/l) than that of the reactors digesting food waste (FW2.6) and cabbage. Both food waste and cabbage reactors had an ammonia level around 1500 mg/l. The high protein content characteristic for meat may have been a contributing factor. The pH and SCOD values of the meat reactor which were about 6.2 and 50000 mg/l, respectively, further demonstrates signs of reactor inhibition.

When sawdust was added together with the meat feedstock, the ammonia levels in the reactor fed on meat was reduced. As revealed in Figure 5.4, the ammonia level of the meat and sawdust mixture only reached 2400 mg/l as opposed to 5000 mg/l when digesting on meat alone. Even so, the soluble COD was still quite high at 45000 mg/l. Organic acids may have still been produced excessively as confirmed by the low pH of 5.0. As a result, the methane production was repressed to only 20%.

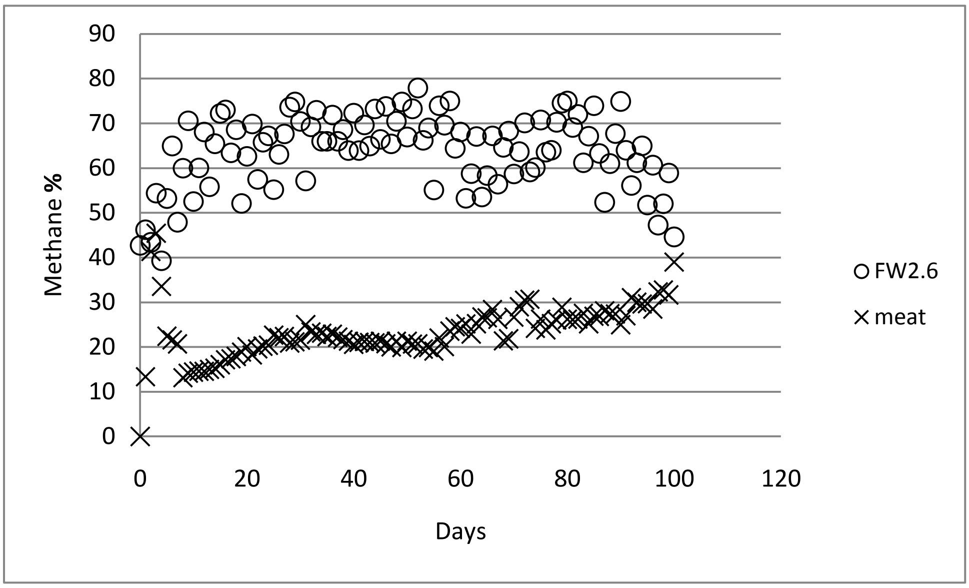


Figure 5.2 Methane percentages of various substrates

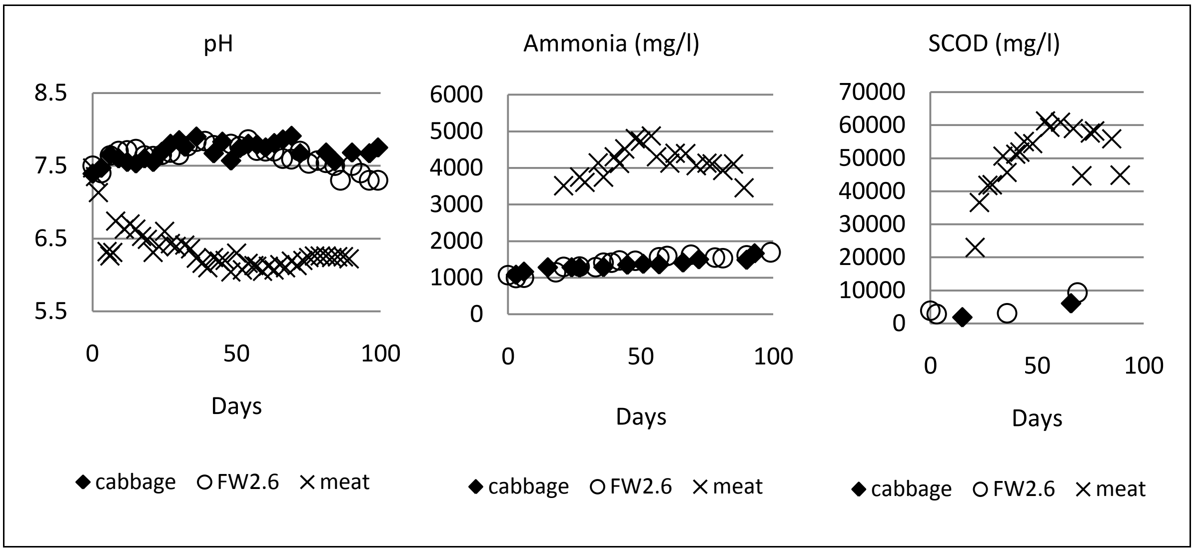


Figure 5.3 The effluent conditions (pH, ammonia and soluble COD) from three reactors fed with substrate of different biodegradability; cabbage, food waste and meat

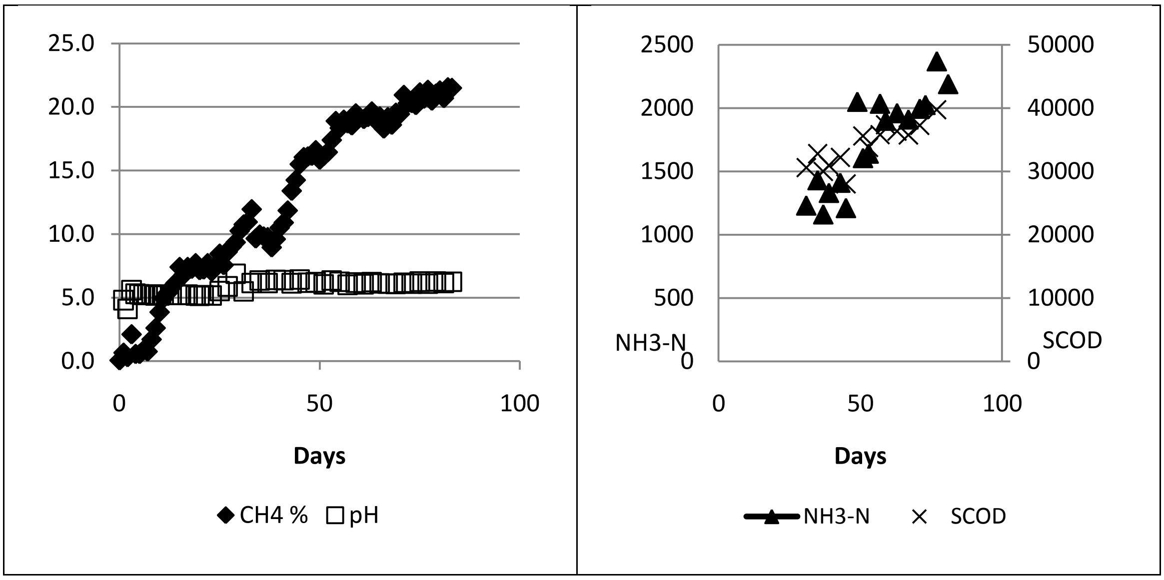


Figure 5.4 The methane percentage, pH, ammonia and soluble COD of meat mixed with sawdust reactor

5.3.1.2 Starting up a healthy reactor

Different start-up procedures have been trialled when initiating the meat, cabbage and food waste fed reactors. These procedures include varying the inoculum's volume at the start, utilisation of another reactor's effluent as well as the inclusion and exclusion of buffer and trace elements in the feed. The result of this preliminary investigation is given in the Appendix 5-a and only the successful start-up strategy is specified here.

When a large amount of seed (15 kg ~ 15 L) was introduced at the start, the methane percentage went to a maximum of 45% at Day 3 (refer Start-up 1, Figure 5.5). This early high methane composition indicates that methane had begun to generate. It could also have been contributed by the large amount of seed (15 kg) introduced at the start. Nonetheless, from then onwards, the methane production declined to an average of 20%. As recorded at Day 5, the pH of the reactor was 6.3 signifying an acidic condition (refer Figure 5.6).

It appears that the initial acid burst production as demonstrated by the increase in TVFA in Figure 5.7 could be one of the deciding factors for a successful reactor start-up. For the purpose of overcoming this initial acid burst, several methods were tested: (i) use of 14 days pre-digested seed and low reactor feeding of every three days (Start-up 2 & 3) (ii) alkali chemical supplementation (Start-up 2 & 3) and (iii) addition of effluent from a healthy operating reactor (Start-up 3). The theory was to allow the VFAs generated through hydrolysis to be converted into methane. Methanogens are commonly known as slow growing bacteria.

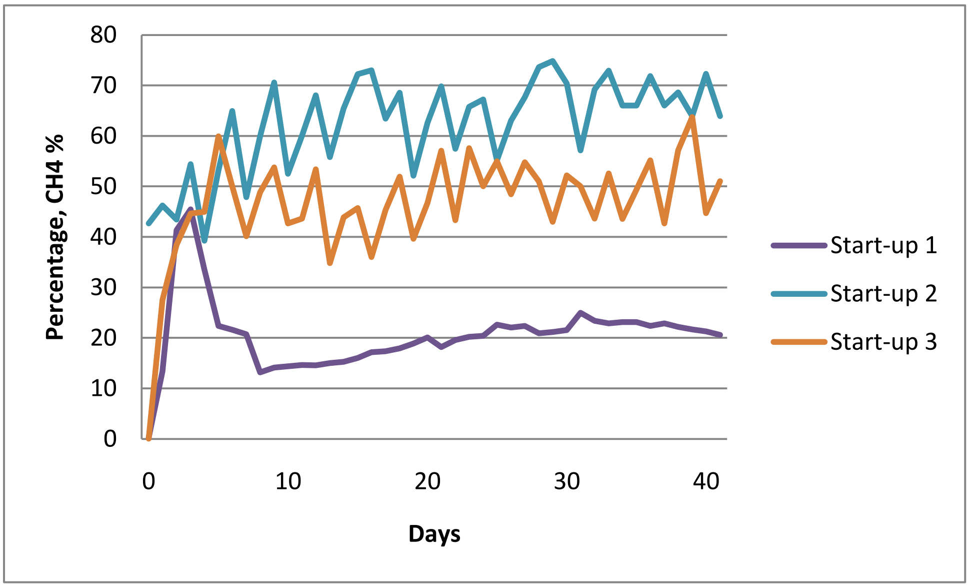


Figure 5.5 Performance of reactors applying Start-up procedure 1 to 3

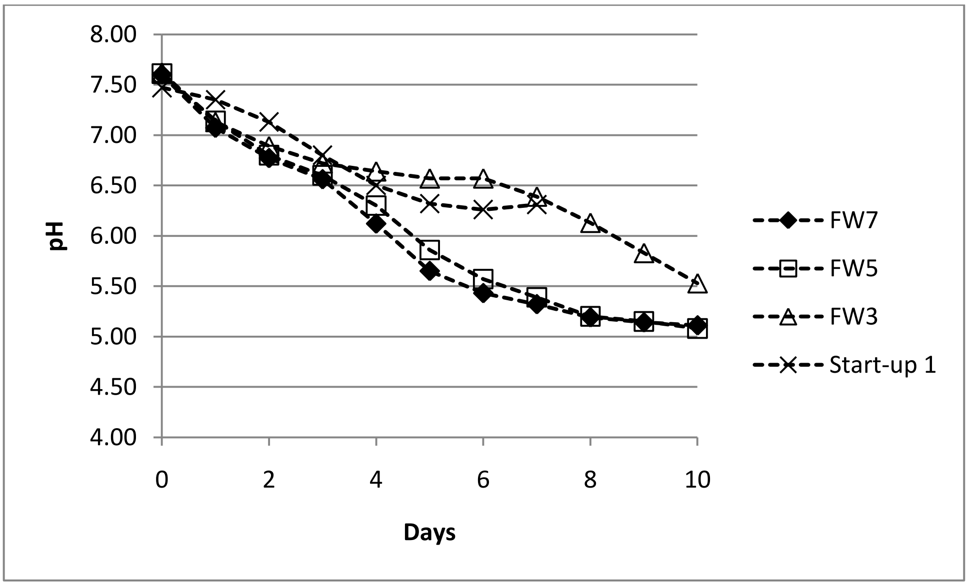


Figure 5.6 The pH trend of reactors FW7, FW5, FW3 and Start-up 1 during reactor start-up

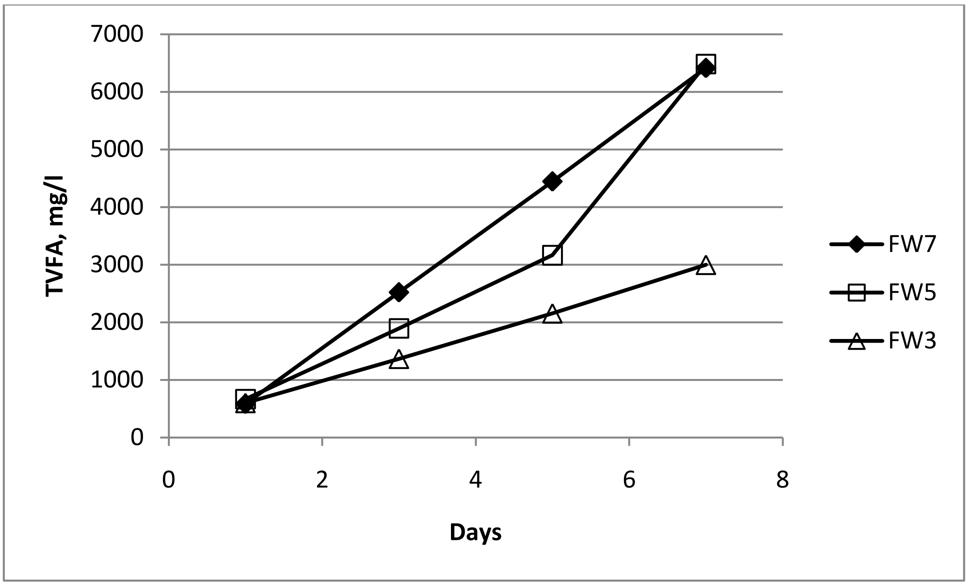


Figure 5.7 The production of TVFA during the early days of continuous reactor operation

Start-up 2 and 3 were beneficial since the acidic conditions observed earlier in Start-up 1, was eliminated (Figure 5.5). Moreover, the seed first adapted to the reactor environment for up to 14 days allowed relative build-up of methane-producing bacteria. Within 4 days of start-up, the reactors with stored seed contained more than 50% CH_4 (approximately 55% for Start-up 2 and 52% for Start-up 3). The corresponding pH values were 7.6 and 7.7, respectively. This positive anaerobic decomposition for both Start-up 2 and 3 continued for 60 days of incubation. A see-saw pattern was evident for both plots as shown in Figure 5.5, coinciding with the 3 day feeding frequency. The CH_4% increased directly after feeding then dropping the day after and more the following day. Unfortunately, feeding every 3 days also means very long hydraulic retention times of 84-90 days.

Seeing the positive effect of the buffer addition and/or effluent from the other reactor, as well as the low feeding rate in preventing the acidic conditions to set in, a similar test was repeated. This time reactors at similar OLR of food waste were compared with (Start-up 5) and without (Start-up 4) the buffer addition. On top of that the reactor feeding was done daily.

Again, the reactors which started on seed without any supplementations failed within 4 days. As shown by Figure 5.8, the FW7 reactor peaked at 34.9% CH_4 before declining onwards on Day 4. The FW5 also peaked at 31.4% CH_4 and then drop at Day 4, while the FW3 reactor sustained the highest CH_4% of 45.4 on Day 7 before failing at Day 14. When buffer solution and trace elements were added, a more positive outcome was attained (refer Figure 5.9). The 7 OLR (FW7) reactor still failed along with the 6 OLR (FW6) reactor despite the supplements. However, the other lower organic loading rates of 5, 3 and 1.5 g VS/L. d. was still going active until the end of the incubation period. These reactors generally took 10 days to reach steady state at which the average methane content was at 60%. As feeding was performed daily, this run also proved it is unnecessary to conduct feeding of every 3 days as was done in Start-up 2 and 3. As a result, the HRT was cut short to just 30 days.

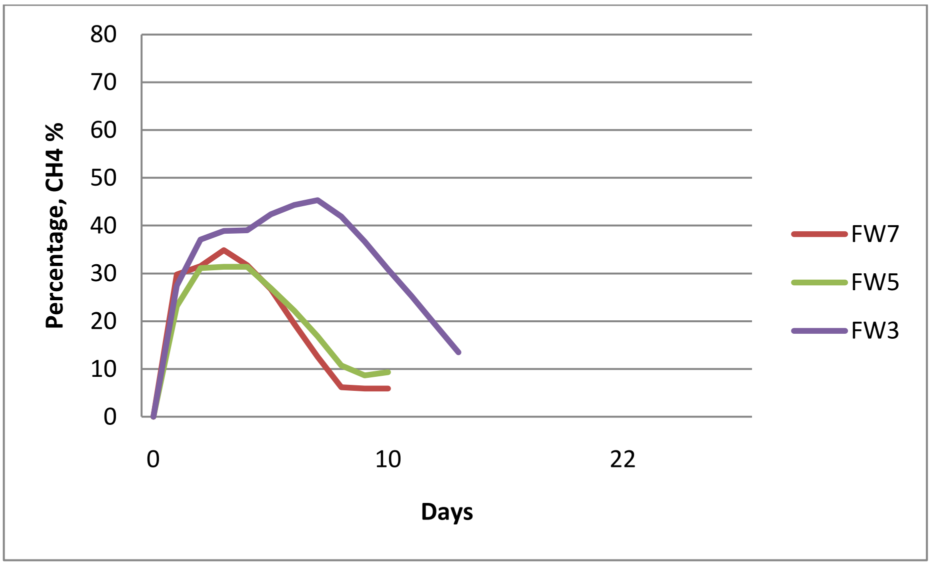


Figure 5.8 Performance of reactors using Start-up 4

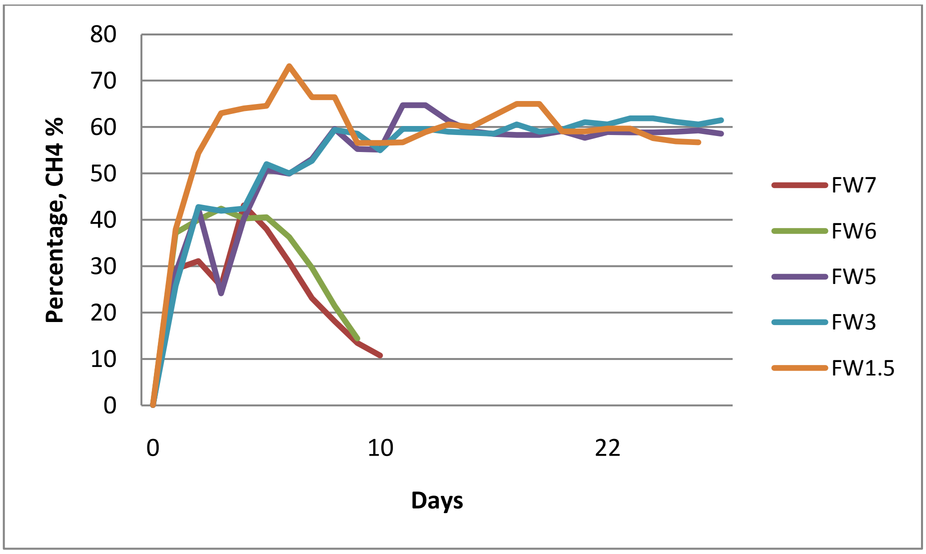


Figure 5.9 Performance of reactors applying Start-up 5

5.3.2 Thresholds of reactor stability

One of the most important aspects when operating an anaerobic digester is to understand whether the system is healthy, stressed or inhibited. This is so early detection can be done and intervention and solutions performed accordingly. A healthy system is defined when a successful methane generation is achieved at steady-state, between 50-70% methane composition in the biogas. Where methane is barely being produced, for example 15-25%, it is indicative of an unhealthy anaerobic process. A methane percentage above 50% is selected to indicate anaerobic decomposition and methane production had begun and is performing healthily. After running several reactors with different feedstock and loading rates, the following threshold values for identification of a healthy and unhealthy process are suggested (refer Table 5.4). The argument for this threshold is given in the subsequent text.

Table 5.4 Suggested thresholds for indicators of reactor stability (values compiled from the continuous digestion of various substrates; meat, food waste and cabbage from this research)

Parameter	Healthy system	Unhealthy system
Methane percentage, %	> 50	< 50
Ph	6.9 – 8.0	< 6.5 and/or dropping from 7.0
Alkalinity, mg/l $CaCO_3$	< 2000-5000	> 8000
Ammonia, mg/l	< 1000	> 1000-2000 pH dependent > 4000
TVFA, mg/l	< 1000	> 6000
Propionic, mg/l	< 100	> 3000
Acetic, mg/l	< 500	> 4000
Butyric, mg/l	< 400	> 3000
SCOD, mg/l	< 10000	> 20000

5.3.2.1 pH

Figure 5.10 (a) shows that healthy reactors would have a pH value between 7.0 and 8.0. Instead, a much lower pH between 6.1 and 6.3 will result in unhealthy methane production with just 15-25%. At this low and acidic pH, any means for resurrecting the unhealthy reactors will be fruitless. Even so, if pH of a reactor starts to decline from pH 7.0, then it is a sign of a stressed condition. At this point, early intervention can help turnaround the situation. Identifying the exact problems and addressing them quickly before further decline to pH 6.5 is the crucial factor that decides the continuation of the stressed reactor. As can be seen in Figure 5.10 (b) pH decline (recorded with the FW2.6 reactor after 100 days of operation), can occur within six days if left untreated.

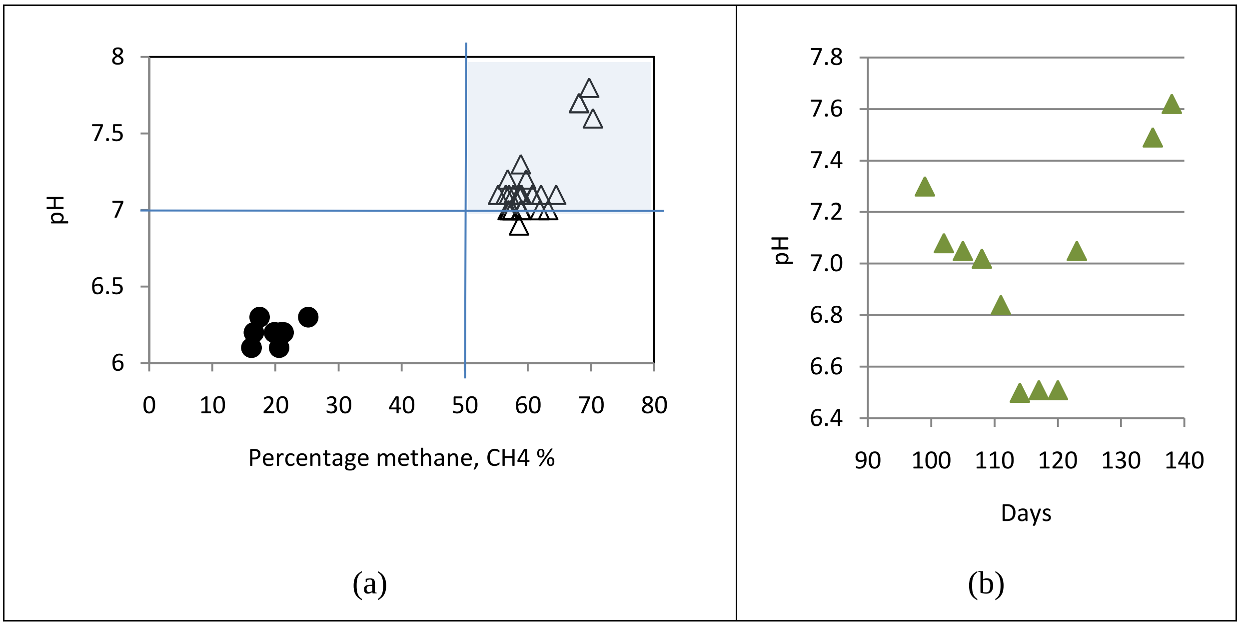
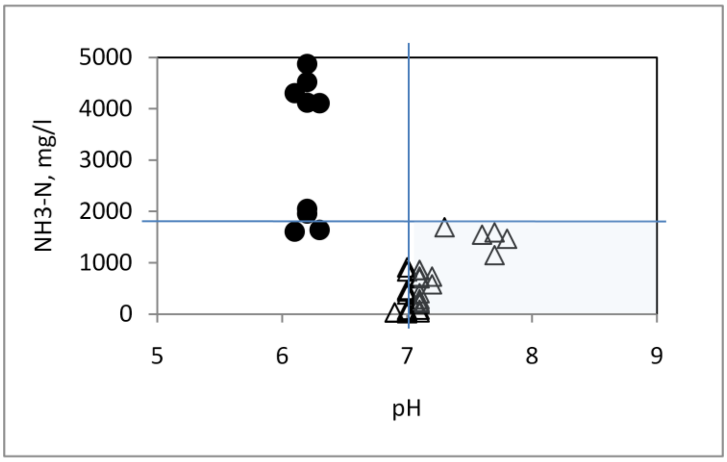


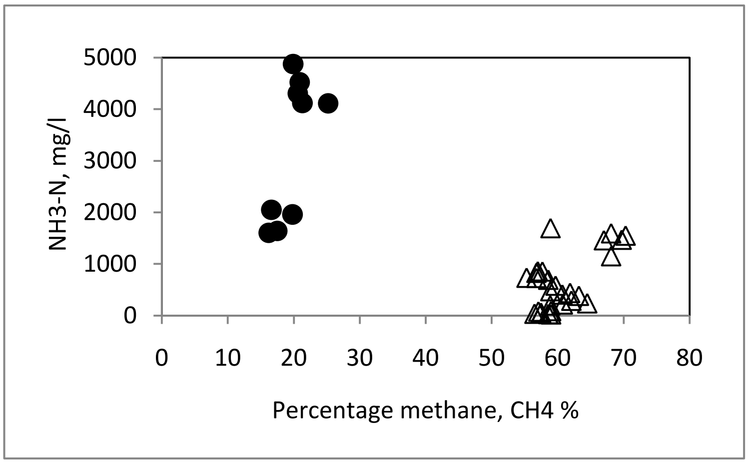
Figure 5.10 (a) Relationship between CH_4% and pH for different reactors (b) pH decline for FW2.6. The shaded area represents a healthy pH range

5.3.2.2 Ammonia

A reactor would most definitely be unhealthy if ammonia is present in excessive amounts between 4000-5000 mg/l. When the ammonia dropped significantly to less than 910 mg/l the reactor is at a healthy state. The ammonia threshold becomes shady when the concentration is between 1000 mg/l to 2000 mg/l. This is because within this range the deciding factor for a reactor condition is also influenced by the reactor pH (refer Figure 5.11 (a)). A methane percentage of 50-70 % (refer Figure 5.11 (b)) was achieved when ammonia was between 1100 to 1700 mg/l at pH around 7.5. A similar concentration of 1600-2000 mg/l at low pH of 6.5 average is likely to be unhealthy, giving only 20% methane.



(a)

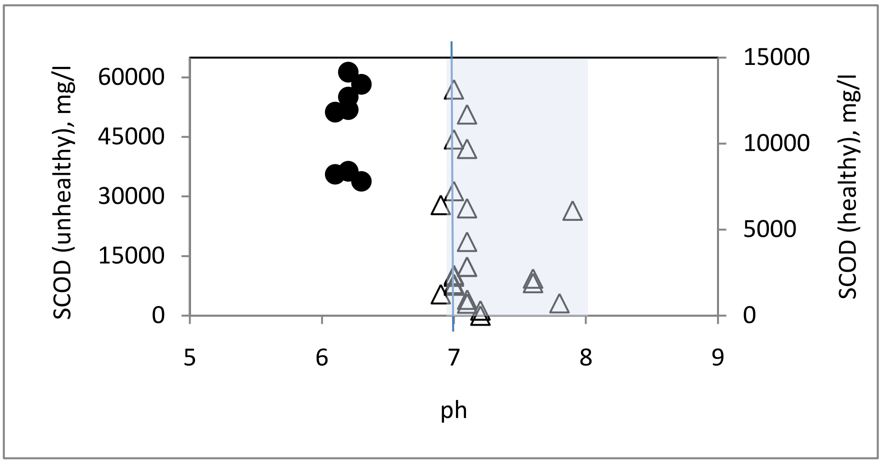


(b)

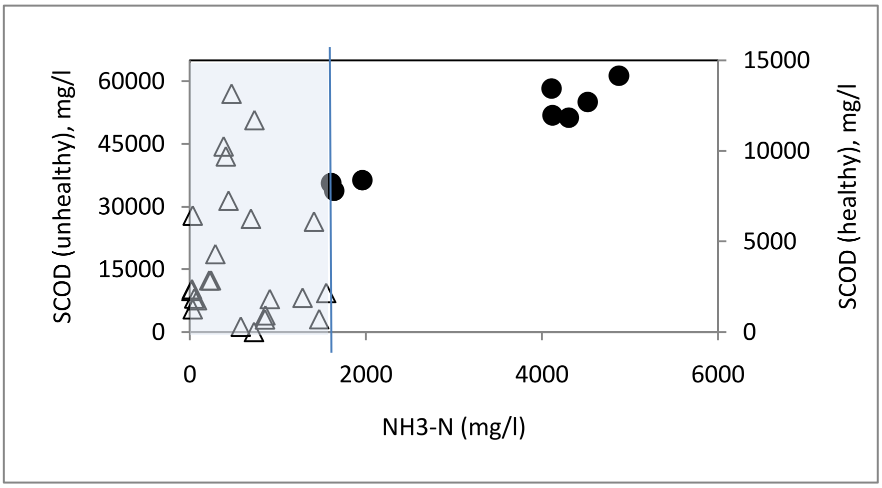
Figure 5.11 Relationship between NH_3-N with (a) pH and (b) CH_4% across various reactors

5.3.2.3 SCOD

Figure 5.12 points out that the soluble COD level for unhealthy system is extremely high, around 35000 to 55000 mg/l. The excessive soluble COD of more than 35000 mg/l in the reactor effluent indicates that there are still lots more organic matter not being converted into methane. The acidic pH close to 6.0 in Figure 5.12 (a) supports this notion. The reactor environment becomes healthy when SCOD is less than 14000 mg/l. These thresholds were obtained for FW2.6, cabbage, FW5, FW3 and FW1.5 as shown on Table 5.5. Within this healthy SCOD range, the pH is neutral at an average of 7.5 with an ammonia concentration less than 1700 mg/l (refer Figure 5.12 (b)).



(a)



(b)

Figure 5.12 Relationship between SCOD and (a) pH and (b) NH_3-N for different reactors. Note different y-axis for healthy and unhealthy SCOD

Table 5.5 The SCOD values corresponding to the methane percentages of a presumably healthy reactor

Condition	CH_4 (%)	SCOD (mg/l)
FW2.6	70	3100
	70	9350
Cabbage	46	1900
	49	6100
	51	10850
FW5	59	13150
	61	9700
	57	2275
FW3	62	7250
	61	2850
	59	1250
FW1.5	60	300
	57	1825

5.3.2.4 VFA

A propionic acid below 600 mg/l in the reactor can warrant a healthy process, whereas a concentration above 3000 mg/l is usually indicative of an unhealthy system. The acidic reactor pH of less than 6.5 with the latter further confirms that the reactor has likely failed (Figure 5.13).

A large proportion of propionic acid in the range of 20-30% is present in the meat reactors compared to the food waste which contained less than 10% (refer Figure 5.14). This translates to an average propionic acid of 5000 mg/l in the meat reactor, 3200 mg/l for meat and sawdust reactor and 70 mg/l in the FW5 reactor. The propionic acid was almost constant throughout the digestion period for the meat and meat and sawdust reactor, fluctuating by 9-12% of its average concentration. The propionic acid evolution was more erratic for the food waste digestion. In the beginning the FW5 reactor saw propionic acid barely present before starting to accumulate to 120 mg/l at Day 23, then dropping to 30 mg/l at Day 44 and rising again at Day 42 etc. No particular pattern of propionic acid production and uptake can be distinguished here. That said, it was thus uncertain whether the propionic acid behaviour could be a sign or contributor for a stressed process.

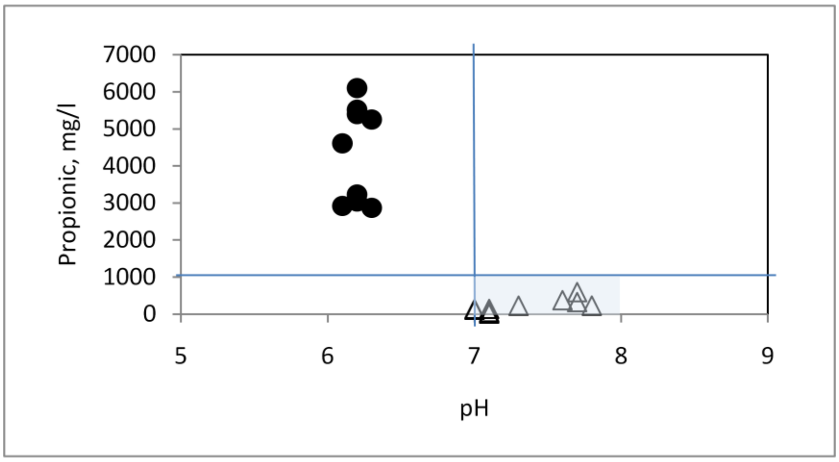


Figure 5.13 Relationship between propionic acid and pH for various reactors

For the food waste reactor, butyric acid was the major fraction making up 58% (roughly 340 mg/l) of the total VFA. Butyric acid in the meat reactor was about 36% of the TVFA but at an elevated concentration of 7800 mg/l. The lowest fraction of butyric acid was found in the meat and sawdust reactor generating 27% of the total VFA equating to 3100 mg/l.

The fraction of SCOD in the form of VFAs was estimated by converting the VFA concentrations to COD equivalents. Conversion factors of 1.067, 1.514, 1.818 and 2.039 as shown in Table 5.6 were used for acetic acid, propionic acid, butyric acid and valeric acid, respectively, obtained from the simple oxidation reactions of VFAs. In general, the VFA concentration correlates with the soluble COD as depicted in Figure 5.14. The VFAs converted to the equivalent COD was within 9-25% for FW5, 43-51% for meat and sawdust and 50-71% for meat digester of the total SCOD measured for each digester. A better correlation between these two parameters were seen after 50 days of reactor operation and at the same time when the methane percentage is almost constant, varying by about $\pm$ 9%-12% from the CH_4% average. The VFA concentration did not seem to encourage or discourage its correlation to SCOD. This is because as shown by Figure 5.14, a poor correlation was dictated when VFA was below 1200 mg COD/l, but became better correlated at a higher VFA of 15,000 mg COD/l. However, the good correlation between SCOD and VFA did not sustain or improve when the VFA increased to 30000 mg COD/l. The reactor pH was also ruled out

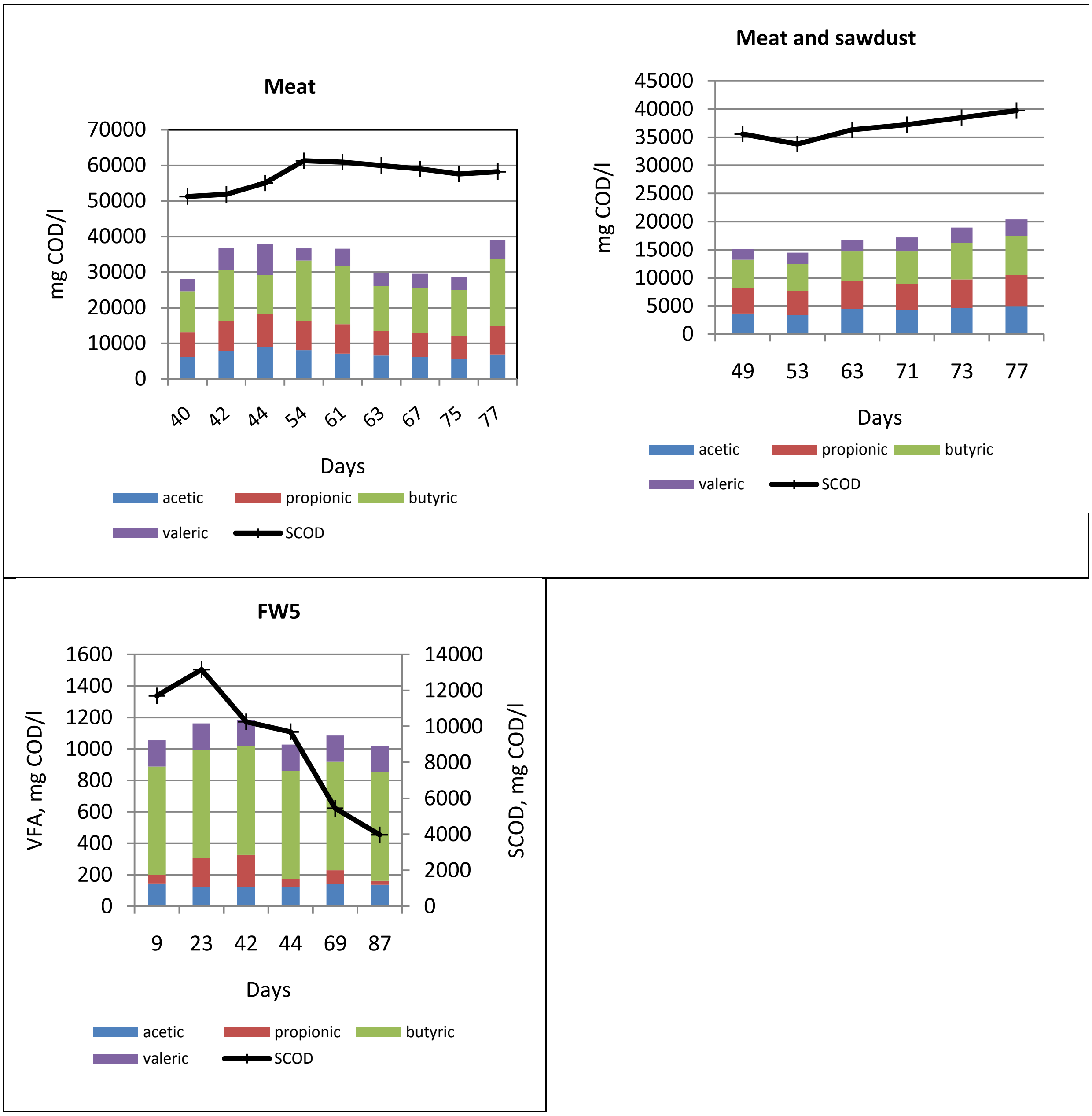


Figure 5.14 The VFA as COD and SCOD distribution in meat and sawdust, meat and FW5 reactors

as an influencing factor as the pH was almost constant for the conditions shown in Figure 5.14. The SCOD can be helpful in validating the VFA readings as well as indicate the performance of the biodegradation process. It is sensible that a reduction in both VFA and SCOD should be expected over the incubation period. Unfortunately, apart from the FW5 food waste, this behaviour was not met by the meat and sawdust supplemented reactors.

Table 5.6 Theoretical COD conversion factors for VFAs

VFAs	Oxidation reactions of VFAs	Conversion factor (mg COD/mg VFA)
Acetic	$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_20$	1.067
Propionic	$2CH_3CH_2COOH + 7O_2 \rightarrow 3CO_2 + 3H_2O$	1.514
Butyric	$CH_3CH_2CH_2COOH + 5O_2 \rightarrow 4CO_2 + 4H_2O$	1.818
Valeric	$2CH_3CH_2CH_2CH_2COOH + 13O_2 \rightarrow 5CO_2 + 5H_2O$	2.039

5.3.2.5 Alkalinity

As highlighted in Figure 5.15, it was quite difficult to interpret the alkalinity threshold. This is because a healthy and unhealthy condition was seen when alkalinity was both low and high. The reactors having about 4000 mg/l $CaCO_3$ alkalinity were operating healthily, achieving roughly 60% CH_4, while the reactors with 10000 - 13000 mg/l achieved 60-70% CH_4. Not only that, the reactors which recorded alkalinity levels quite close to the 8000-15000 mg/l range was not healthy, obtaining a lower methane of around 15% instead. It has to be remembered that some of these reactors; the meat based reactors and FW2.6 were buffered occasionally during the incubation period (refer Figure 5.16, the cross ‘x’ symbols indicates buffering events). These are when buffer solution (100 ml phosphate buffer) is added to the reactor following a plunge in effluent pH. As can be seen, there are times (before Day 50 for both graphs) when the alkalinity responds to the added buffer as evident by an increase in alkalinity. However, more often, this was not necessarily the case as alkalinity appeared unchanged (after Day 50 for both graphs) after buffering. In addition, as shown by Figure 5.17 the ammonia content with some of the reactors may have an effect to the alkalinity measurement, where high ammonia content saw an increase in the alkalinity concentration. These behaviours seemed to point out that alkalinity measurement may not be as responsive or reliable to indicate changes in the reactor environment as commonly presumed. Perhaps alkalinity is more valuable if just examining one substrate, but less valuable if comparing between substrates.

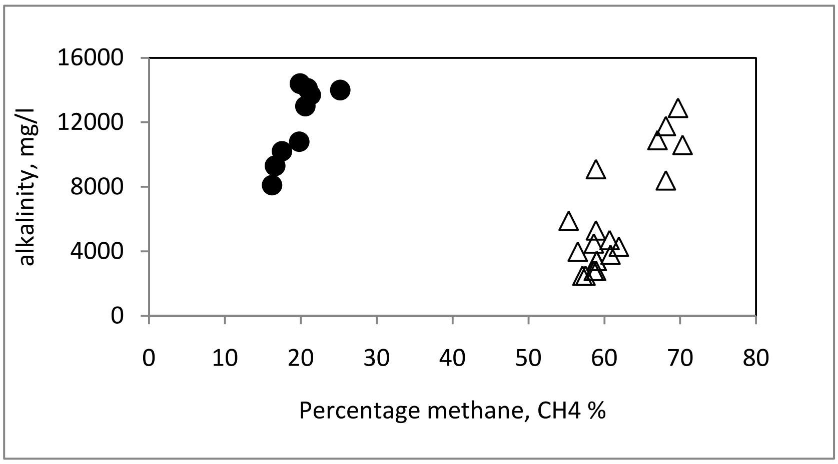


Figure 5.15 Relationship between alkalinity and CH_4%

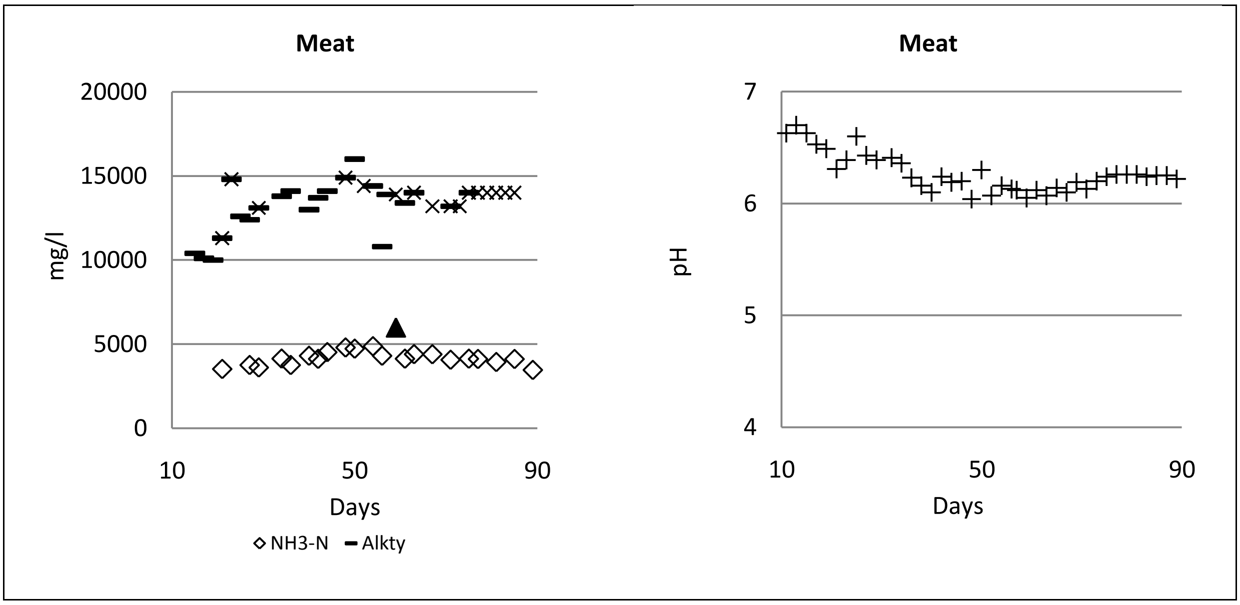


Figure 5.16 The relationship between ammonia and alkalinity concentration in meat reactor. The x symbol denotes buffering occasions where alkali chemical is added during reactor feeding. The triangle represents 'high' hydrogen levels (hydrogen could be above 30,550 ppm, 3%) as measured by the Gas Analyzer

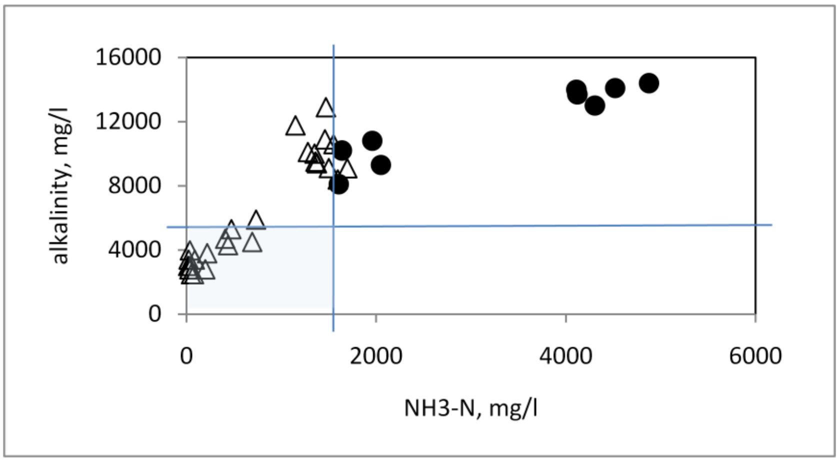


Figure 5.17 Relationship between alkalinity and NH_3-N across the many reactors

5.3.3 Factors and solutions for process imbalance

Here, the occurrence of reactor deterioration is shown, detected based on the threshold values highlighted in Table 5.4. The suspected factors that might have led to the negative condition are revealed and the solutions presented.

5.3.3.1 Washout

After running a healthy food waste reactor for more than 90 days, a drop in methane production occurred. Methane until 90 days digestion was 75% but was just 45% at 100 days. The data in Table 5.7 showed that ammonia was not toxic to the process as the concentration had been relatively steady at 1500 ($\pm$ 200) mg/l throughout the incubation period. On the other hand, the rising TVFA, SCOD and the drop in pH showed signs of a reactor that is losing stability and becoming stressed. After 99 days, the VFA was 970 mg/l and pH was 7.3. The VFA concentration more than tripled to 3000 mg/l VFA after 105 days with a 7.1 pH. The SCOD which rose from 9400 mg/l at 60 days to 26000 mg/l at 120 days further confirmed a troubled reactor. Since ammonia was ruled out as a possible factor, the lack of methane generation coupled with a rising acidic conditions seems to suggest washout of micronutrients as a factor. Diminishing micronutrients seems to upset and stunt the growth of methanogens.

Table 5.7 The FW2.6 reactor condition before and after treatments

Day		NH_3-N (mg/l)	SCOD (mg/l)	TVFA (mg/l)	pH
36	Before treatment	1400	3100		7.8
69		1600	9400		7.6
78		1600		970	7.6
99		1700		970	7.3
105		1400		3000	7.1
111		1700		2100	6.8
120	Treatment 1: Other effluent added	1500	26000	2200	6.5
150			26000		7.6
162		1600	23400		7.2
174	Treatment 2: Trace element added	1300	19400		7.4
186		1200	9500		7.5
193		1200	6700		7.6

To tackle the above problem, effluent from another reactor digesting on cabbage was included when feeding the FW2.6 reactor from 114 days (refer Figure 5.18). Immediately after, a rise in both the methane percentage as well as the pH was observed. This positive response seemed to agree with the earlier notion that washout led to a decreased reactor performance. Because the cabbage effluent may have provided extra micro-organism and nutrients, a turnaround of the troubled FW2.6 reactor was seen. However, looking at Table 5.6, in spite of the positive effect of effluent addition, when added continuously, there are also risks of transferring any ill effects from the other reactor. For example, the high TVFA of the FW2.6 reactor sustained even after effluent addition (the VFA after 111 days was 2100 mg/l but was the same at 120 days). The SCOD too remained about 25000 mg/l from 120 to 162 days. Eventually, a drop in reactor performance starts again.

Building on this observation, at 171 days, the FW2.6 reactor was supplemented with 5 ml trace element solution at each feeding. As shown by Figure 5.18, the trace elements addition was effective since a rise in methane percentage and pH was observed. Unlike the above effluent addition, the SCOD of the FW2.6 reactor dropped significantly when trace element was added. No data was available for VFA, but, a fall in SCOD should coincide with a drop in VFA (based on Section 5.3.2). The rise in pH confirmed that the acidification effect has been reversed. As at 193 days incubation, the SCOD was 6700 mg/l, pH 7.6 and methane 71%.

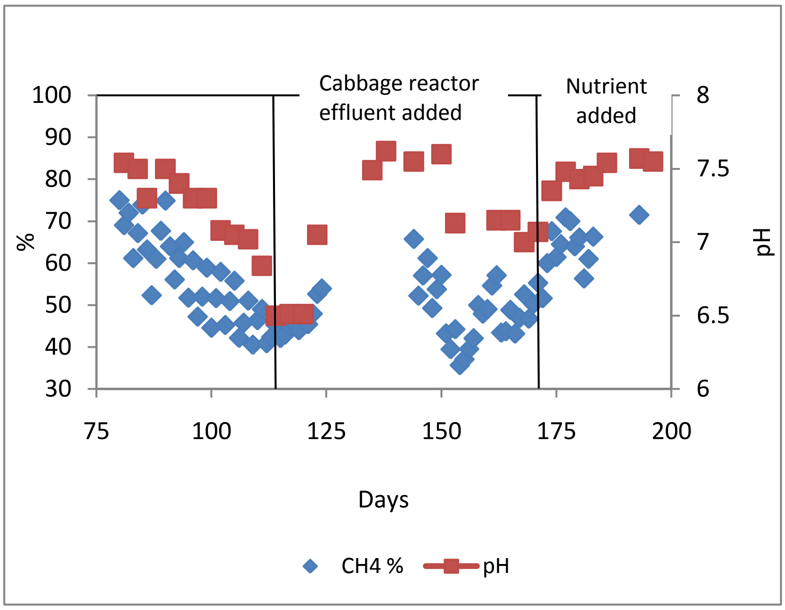


Figure 5.18 The occurrence of washout after 100 days, and the methods to recover the FW2.6 reactor

5.3.3.2 Inhibition

The meat reactor produced between 40 to 50% CH_4 until 110 days, before the methane production began plummeting to less than 21% after 127 days (refer Figure 5.19). The pH too fell from 6.7 after 108 days to 6.1 after 126 days. The methane percentage and pH readings show signs of an inhibited reactor which was also confirmed by the high SCOD of 50000 mg/l (refer Table 5.7). During the meat reactor operation, microbial or nutrient washout has not been thought to have led to reactor failure. It was only later (when meat reactor was no longer operating) such washout possibilities were learnt, particularly when some reactors recovered following the addition of trace element. If not, it would have been informative to see the effect of trace element addition on the meat reactor. Nonetheless, the excessive ammonia content around 4000 mg/l is likely to be the cause of the process inhibition.

As the process may already have been inhibited, it was thought less feasible to try reviving the meat reactor. Therefore, a drastic decision was made to exchange half of the reactor content with deoxygenated water at 128 days digestion. As shown in by Figure 5.19, this decision was ineffective since the pH of the meat reactor only rose to 6.6 which later dropped to pH 6.0 in 3 days. However, diluting the reactor does help cut the ammonia content by half to 1500 mg/l (refer Day 132 Table 5.8). Due to the introduction of water into the reactor which contained no anaerobic micro-organism, the methane content following reactor dilution dropped significantly to 6 and 8%.

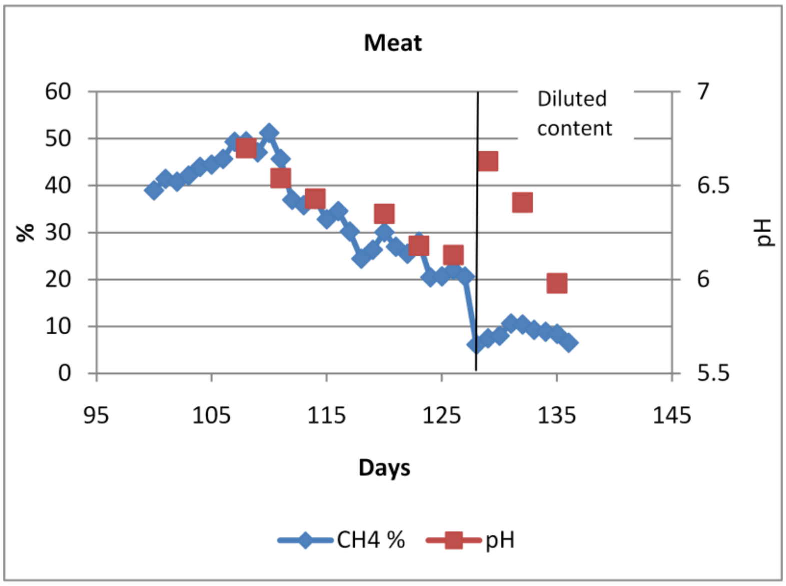


Figure 5.19 The decline of meat reactor performance after 127 days, and effect of reactor dilution

Table 5.8 The meat reactor conditions before and after treatment

Day		NH_3-N (mg/l)	SCOD (mg/l)
85		4110	55850
89		3455	44900
108	Before treatment	3800	52650
111		3635	
123		3330	
126		3880	
132	Treatment: Reactor dilution	1460	

5.3.3.3 Temperature drop

On the 27th day of operation, the temperature controlled room failed and the temperature dropped from 35°C to 23°C. As a consequence, the two reactors, 5 and 3 OLR (labelled as FW5 and FW3, respectively) which were incubated in the room were stressed. This was evident in the methane percentages and pH that fell to 49% and 6.8, respectively from the day before of 59% and pH 7.0 for the 5 OLR (refer Figure 5.20). The 3 OLR recorded a drop to 54% and 6.9 pH from the previous day reading of 61% CH4 and 7.1 pH.

Two actions were carried out to regain the reactor performance; by reducing feeding and adding effluent from another reactor. Figure 5.20 showed the reactors act differently to the same treatment methods. The lower OLR of 3 g VS/L. d. took only 1 week to recover, solely by lapsing the feeding by one day. After 33 days digestion, the reactor recovered with methane 68% and pH 7.1. On the other hand, the higher OLR of 5 g VS/L. d. took twice as long to bring the methane back to 65% and 7.0 pH (after 41 days diestion). This positive effect was achieved by first delaying the feeding. This treatment was performed for 1 week. Unlike the FW3 which responded successfully, no improvement was observed for the FW5 reactor. Following this, the effluent from the FW3 was added into the FW5 feeding. After doing this for another week, a positive recovery as shown in Figure 5.18 (FW5) was obtained.

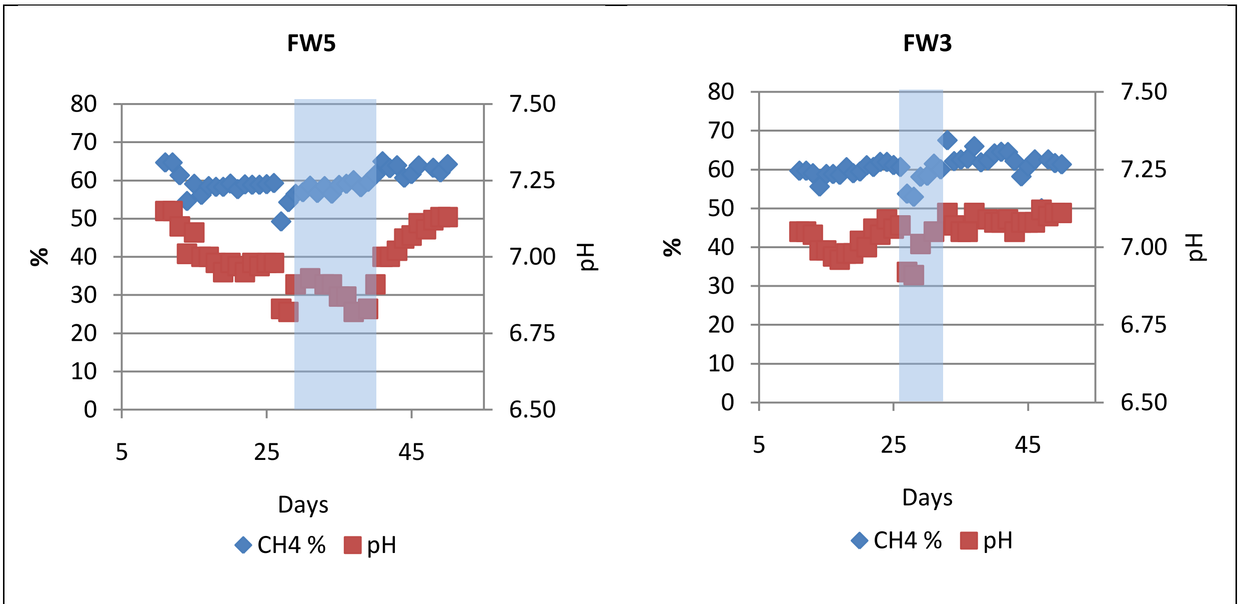


Figure 5.20 The decline of reactor performance due to temperature drop on day 27, and the recovery (shaded) following treatment

5.4 DISCUSSION

5.4.1 A healthy reactor

5.4.1.1 Reactor performance

The healthy reactors; mainly the reactors labelled as FW2.6, FW5, FW3 and FW1.5 all recorded methane contents about 50-70%. At methane content above 50%, the gas can be used for combustion, heating and other forms of energy. Reactors with low methane percentages give an initial indication of the inhibition of methanogenic bacteria (Chynoweth and Isaacson, 1987). Other researchers digesting on food wastes also achieve more than 50% methane composition. Bouallagui et al. (2005) achieved 58-62% methane, Stentrom et al. (1983) obtained 50-60% CH_4 while Mata-Alvarez (2003) reported typical values as 50-75%.

The methane percentages were always consistent at every reading. However, in two separate occasions, the methane percentage suddenly dropped followed by a sharp rise in carbon dioxide. These occasions were; first, the reactor accidently exposed to oxygen and second, sudden temperature drop. The quick response of the biogas content was advantageous and enabled quick detection of reactor problem. The only downside to the parameter determination would probably be more of an instrumental issue such as sensor malfunction and moisture ingress with the Gas Analyzer than anything.

5.4.1.2 Start-up

Start-up is important in establishing a proper microbial community (Angelidaki et al., 2006). Poor start-up in biological treatments can lead to prolonged period of acclimation and ineffective removal of organic material. When the reactors were initiated with predominantly substrate (14 kg wet weight) and just 400 ml of seed (Start-up 1 and 2) (result in Appendix 5-a), the process failed as evident by the pH values and methane which were below 6.0 and 10%, respectively. In such low seed volume, the methanogenic population may have been insufficient to prevent VFA accumulation causing the imbalance between process of hydrolysis/acidogenesis and methanogenesis (Vavilin et al., 2004). The importance of ‘methanogenic activity’ in removing VFA at all time cannot be stressed enough (Angelidaki and Sanders, 2004). This may have in fact contributed to the positive outcome from using a larger inoculum volume of 14 L in Start-up 1. Here, a methane percentage of 45% was reached. Unfortunately, this was not sustained and methane production started declining to 20% from Day 4 onwards. An accumulation of VFA and/or pH toxicity causing digester stress was suspected following a low pH of 6.3. It has been reported that most methanogenic bacteria function in a pH range between 6.7 and 7.4, and that the rate of methanogenesis may decrease if the pH is lower than 6.3 (Lay et al., 1997).

Angelidaki and Sanders (2004) highlighted that hydrolysis depends on the concentration of the substrate; therefore, the highest production of VFA is to be anticipated in the first day after incubation. Methods to calm the initial acidic bursts during reactor initiation may be necessary to ensure that the digester can proceed to the steady-state stage. As shown by Start-up 2, 3 and 5, an average of 65% CH_4 was obtained after 10 days of start-up period. The reactors (Start-up 2, 3 and 5) were buffered for 9 straight days. This nine day buffering period includes the first 4 days for these reactors to reach 50% methane and another 5 days were allocated to check whether these reactors would sustain this minimum 50% methane production. The reason was to ensure that a strong population of methane generators have developed (based on >50% CH_4) that would be able to convert the produced VFAs. Therefore, buffering can be stopped with surety that the anaerobic system within these reactors is capable of self-regulation. The quick high methane production (where 65% methane is being produced less than 11 days) could have also been a combination from adding trace elements as well. In Start-up 5, despite the buffering, the reactors which were operating at a higher OLR of 6 and 7 g VS/L. d failed within 5 days. The pH of the reactors was 6.4 and methane was about 40%. The results indicate that although buffering was provided, reactor failure could not be prevented if the OLR was too high.

5.4.2 Thresholds of reactor stability

5.4.2.1 pH

pH close to neutral values has been quoted by many researchers to be best for anaerobic digestion system. Mata-Alvarez (2003) suggested the pH range of 6.5-7.5, Chynoweth and

Isaacson (1987) between 7.0-8.0 while 6.8 to 7.4 by Grady et al. (1999). This research agrees since high methane percentages were seen at pH 7 – 8 (refer Table 5.4). Instead, low methane percentages were usually the case when pH is less than 6.5. As shown by Figure 5.10 (b) another sign of a distressed reactor is when the reactor pH falls by 0.15 – 0.25 every day, especially so if initial value is close to 7.0. This means it takes between 2 to 4 days before the reactor pH drops below 6.5.

pH measurement itself is very straightforward and usually involve a data logger and a probe. Because simple instrumentations are occupied the pH meter is available both as a desktop or a handheld portable device. This makes pH measurement virtually applicable everywhere and most advantageous for measuring conditions real-time. The calibration method also is very simple by resetting the device using standard calibration solutions. The measurements are therefore confidently precise plus there is no compromise in time like other parameters that require factory calibrations. This research also found the pH measurement to be useful across various substrates such as food waste slurry, meat slurry, cabbage slurry and digested sewage sludge. These samples were measured as is without any need for sample pre-treatments.

Any disadvantage to the pH measurement would be more of the sample condition. For example, meat substrates leaving greasy residue which could foul the pH probe if left uncleaned. The probes were rinsed with methyl solution and deionised water before storing. Another issue is due to small pieces of food waste or sawdust stuck in the probe. This is usually solved by poking out the stuck materials using paper towels. pH data is also reliable if reactor content is exchanged every day or two, considering in this research, the reactor pH is monitored from the effluent sample. The pH data becomes less reliable following a reduced feeding and wasting frequency or in batch systems since less pH measurements is possible.

5.4.2.2 Alkalinity

The alkalinity concentration between 2000-5000 mg/l was found to be representative of a healthy operation. Researchers generally reported typical values of alkalinity in anaerobic digesters in the range of 1000-5000 mg/l $CaCO_3$ (Chynoweth and Isaacson, 1987; Grady et al., 1999; Stenstrom et al., 1983; and Mata-Alvarez, 2003). Castillo M. et al., (2006) found process is stable at total alkalinity of 4500-5500 mg/l $CaCO_3$. An alkalinity above 8000 mg/l may be an indication of a stressed reactor. As discussed earlier, excessive ammonia can elevate the alkalinity concentration.

Alkalinity was determined by titration method where acid solution is added into the sample until pH 4.5. The samples were pre-treated by centrifugation, filtration and dilution in order for measurement to fall within range. These means probably 10 to 15 minutes were wasted before sample could be tested. A rise between 0.59 and 1.39 in pH units were usually observed by the time sample was ready to be tested. The effect of time lag to the sample pH for alkalinity testing has already been discussed in Chapter 3. Nonetheless, sample pre-treatment cannot be avoided as measurement can be affected by such interfering substances as soaps, oily matter, suspended solids, or precipitates that can contribute to imprecise titration endpoints (Chynoweth and Isaacson, 1987).

5.4.2.3 VFA

This research measured the acetic, propionic, butyric and isovaleric acids which are summed and termed as total volatile fatty acids (TVFAs). Volatile acids fermentation products such as acetic, propionic and butyric acids are formed by ‘acid-forming’ bacteria through the fermentation of hydrolytic products (Chynoweth and Isaacson, 1987). A total VFA of 1000 mg/l and less seemed to signify a stable anaerobic digestion (refer Table 5.4). This is comparable to the recommendation by Angelidaki and Ellegaard (2005) of 1000-2000 mg/l, less than 1000 mg/l by Canovas-Diaz and Howell (1988) and 100-1400 mg/l by Stenstrom et al. (1983). Values above these usually are signs of a stressed reactor. The reactor could be inhibited if TVFAs exceed 6000 mg/l as shown by Table 5.4. Wang et al. (2002) did not observe methane when the TVFA increased to over 13000 mg/l.

Kayhanian and Tchbonoglous (1993), Mtz-Viturtia et al. (1995) and Raposo et al. (2006) established that for healthy anaerobic digestion, acetic acid is the highest followed by propionic and small quantities of other acids. High concentration of butyric acid was reported to be toxic to methanogens (Kayhanian and Tchbanoglous, 1993). This is conflicting to the findings of this research where for a healthy system, butyric is generally predominant and propionic the least (refer Section 5.3.2). Clarke et al. (2007) stated that propionate decrease with the onset of methanogenesis. This research found thresholds of 100, 500 and 400 mg/l, respectively for propionic, acetic and butyric acids as indicative of a healthy operation. This is in accordance to the results by Calli et al. (2005) where propionic acids above 200 mg/l and acetic acid above 400 mg/l were inhibiting, whereas Raposo et al. (2006) suggest a higher acetic acid threshold of 1400 mg/l. Clarke et al. (2007) saw stabilization of the reactor when butyric acid fell from 5000 mg/l to 100 mg/l.

On the other hand, all three major VFAs (acetic, butyric and propionic) seemed to be high and in equal proportions when the reactor is unhealthy (refer Table 5.4). These acids increased roughly by ten times in an inhibited reactor; 3000 mg/l for both propionic and butyric acids, and 4000 mg/l for acetic acid. Wang et al. (2002) reported high concentrations of acetic, butyric and propionic acid in decreasing order; 4400 mg/l, 4300 mg/l and 2800 mg/l, respectively, when methane was absent.

The contradictions observed between this research and the work by other researchers with regards to the predominant VFAs, was probably due to population selection according to type of substrate (Demirer and Chen, 2004). Another possibility was that VFA may not have been the cause, rather acidic pH was the inhibitor factor (Vavilin et al., 2007; and Grady et al., 1999). Grady et al. (1999) stressed that inhibition caused by VFAs will be of little concern as long as the pH remains within the normal range for the growth of methanogens (6.8-7.4). This probably explained why for this research, although butyric was high in the healthy reactor, the system was operating at a stable condition following the neutral reactor pH.

The VFA readings are very insightful in showing signs the reactor is edging towards a stressed or inhibited system. For example, this research showed a high presence of propionic, and an overall escalating VFA, when the reactor is deviating from a healthy condition. This is because under a healthy digester, there is a balance between the amount of acids produced and the amount removed through product conversion by the ‘methane-forming’ bacteria (Chynoweth and Isaacson, 1987). In addition, monitoring the individual volatile acids is helpful as further measure of digester stability. However, despite its obvious advantage in reactor monitoring, constant instrument degradation and malfunction wears down on the experimental timeline. Tedious sample preparation and compulsory quality checks makes this analysis less attractive especially if dealing with 'dirty'slurry like sample like food waste. Roughly half an hour is required to centrifuge and filter a sample to 0.22 µm. This process can add to the analysis costs considering a couple of filter papers sized 0.45 and 0.22 µm is used to clean one sample.

5.4.2.4 SCOD

If the reactor soluble COD is below 10000 mg/l, the reactor is likely to be healthy. Meanwhile, the reactor may be stressed when SCOD exceeds 20000 mg/l. As shown by Figure 5.14, the SCOD evolution is comparable to the VFA, suggesting the suitability of the former to indicate degree of anaerobic degradation as well as signs of reactor condition. This is in line with the observation by Ince et al. (1995) where a significant decrease in the COD removal efficiency of the system or even system failure followed a sharp increase in reactor TVFA. Clarke et al. (2007) too observed a close agreement between measured soluble COD and theoretical COD equivalent of the VFAs.

The relationship between COD and VFAs has also been discussed by other researchers. Calli et al. (2005) saw an increase in the acetic and propionic acids when the COD increased. The CODs increased as result of the solubilisation and acidification of the substrate as digestion progressed (Raposo et al., 2006). In the study by Raposo et al. (2006), the shape of the graphs VFA and COD are very similar. Their study found VFA accounted for 53%, 63%, 72% and 78% of the CODs in the various reactors. Bouallugui et al. (2004) recorded 97% of soluble COD as VFA. Our results as shown in Figure 5.14 reported 43-51% and 50-71% of soluble COD is present as VFA in the meat and meat and sawdust reactor. A significantly lower VFA as SCOD of 9-25% was found when the reactor was more stable as in the digestion of food waste at 5 OLR (FW5). It is also evident that VFAs are not the only soluble COD contributing compounds in the reactor, but that some other unidentified substrates which could be associated with metabolic intermediates and unused soluble substrates can be present in large concentrations.

Measuring COD is advantageous when VFA data is less reliable or during GC breakdown. Apart from the usual sample pre-treatment (centrifugation, filtration and dilution) which takes 10 – 15 minutes, the tests itself takes 2 hours to run. However, since the process is automated (the test vials are left in a chamber to digest at 150°C during this two hours) the test period allows the researcher return after two hours to take a 30 seconds reading per sample. The cost for one of the non-reusable vials is $5 NZD, which can be costly with increasing number of samples and test frequency. Even though COD has been touted as a better approximation of organic matter (Chynoweth and Isaacson, 1987) over other parameters like volatile solids, measuring COD for thick slurry-like sample, the sample pre-treatment and its effect on the COD result is sometimes uncertain. This is because, as experienced in this research, when analysing food waste digestate, measurement is limited to the soluble form. This is similar to the work by Lin et al. (1999) and Toorkian et al. (2003), where COD is obtained by measuring the COD of the filtrate collected after filtering the digested sample through a 0.45 μm filter. Sadaka and Engler (2000) found that COD reduction is higher than VS reduction. The higher COD reduction could have been misleading since the highly particulate matter with the manure required multiple sample dilutions for the measurement to fall within range of the analysis.

5.4.2.5 Ammonia

The experiments here established ammonia level less than 1000 mg/l as representative of a healthy reactor. Britz et al. (1988) too recommends ammonia threshold below 1700 mg/l. Kayhanian and Tchobonoglous (1993) stated that maintaining ammonia at or below 600 mg/l will increase methane production and reduce occurrence of ammonia toxicity. Their study also concluded reactors with ammonia close to 1000 mg/l to be operational but was less stable. This is similar to the findings of this research suggesting for ammonia levels between 1000-2000 mg/l the reactor pH should also be taken into consideration to determine its stability. This is because, for the same amount of ammonia, the process would be healthy under neutral operating conditions but stressed if pH reactor is acidic. Finally, this research also established reactor inhibition likely if ammonia is over 4000 mg/l. Calli et al. (2005) also found irrespective of pH, ammonia is toxic when above 3000 mg/l.

5.4.2.6 H_2

When a digester is operating under stable condition, the H_2 was in low concentration (refer Table 5.4). This increased to either medium or later high if the reactor condition is unstable (as shown by Figure 5.16). A lack of H_2 utilizing methanogens is evident by the build-up of H_2 (Clarke et al., 2007). Stenstrom et. al. (1983) also saw H_2 in biogas only in periods of extreme upset. O'Sullivan et al. (2005) recorded H_2 as negligible with concentration total less than 0.1 g SCOD measured as H_2. This does not mean that H_2 was not produced in the digestion but that it is very rapidly converted to CH_4 by hydrogenothropic methanogens. Accumulation of H_2 partially inhibits further formation and consequently more electron sink will be formed, causing imbalances and cessation of methane production (Bouallagui et al., 2005).

Using a gas analyzer, the H_2 detection was very easy. However, due to its poor reporting ability as discussed in the Methodology Chapter, the H_2 measurement using gas analyzer may be less sensitive. As demonstrated by the results in Chapter 3 regarding gas analyzer calibration to H_2 measurement using gas chromatograph, H_2 might be a convincing indicator to help diagnose bench-scale reactors that are becoming unhealthy provided a suitable method is available. The method should be precise enough to measure hydrogen at low concentrations and be available on-site so that any measurements can be done quickly. Mata-Alvarez (2003 p 161) too agreed hydrogen concentration in biogas is low and difficult to determine. Therefore, this measurement is generally absent in industrial application of the anaerobic digestion processes.

5.4.2.7 Relationship between the various parameters

The pH, VFA and alkalinity have been generally accepted by many researchers to be interrelated and serve to depict the global environment in an anaerobic reactor. The change in digester pH, from some disturbance, is a function of the alkalinity. Under stable conditions, the alkalinity is well maintained so that any increase in VFA production can be tolerated with minimal decrease in bioreactor pH (Grady et al., 1999). This relationship helps explains the behaviour of the two stable reactors in this research; the FW2.6 and FW5. In the former, a decrease in VFA was observed, which could be a consequence of their metabolization by the methanogenic bacteria (Mtz-Viturtia et al., 1995). Following this VFA uptake, the alkalinity was restored and pH increased as a result. With the FW5 reactor, the VFA concentration and pH was generally constant, but alkalinity dropped by roughly 50%. The steady VFA signifies a healthy process because VFA is immediately metabolized and a high methane yield could be expected (Mtz-Viturtia et al., 1995). Although the alkalinity dropped to 3000 mg/l, that was sufficient to maintain pH and allow methane production, as evidenced by the high methane percentage of 65%.

Figure 5.16 suggests that instead of stimulating bacterial growth and adding to the buffer capacity, the ammonia became inhibitory in the meat, meat with sawdust and FW2.6 reactors due to its high concentration. Ammonia influences mainly the phase of methanogenesis, with acetate-consuming methanogens more sensitive than hydrogen-utilizing ones (Britz et al., 1988 and Calli et al., 2005). Process instability due to ammonia often leads to VFA accumulation which again leads to decrease in pH and alkalinity (Angelidaki and Ellegaard, 2005; Calli et al., 2005; Chen et al., 2007; and Neves et. al., 2008). This occurrence is more a concern for protein rich substrates and thus explains the same effect observed with the meat and meat and sawdust digesters in this research.

5.4.3 Recommendation of suitable indicators

Based on the above discussion, this research recommends pH and methane percentage as the important indicators of reactor condition. In Section 5.31 and 5.3.2, a stable reactor is indicated by pH of 6.8-7.2 at 50% CH_4 and pH of 7.5-7.9 for 70% CH_4. A lower pH and methane percentage signified unhealthy reactors, reporting pH and methane values of 5.0-6.2 and 20-40%, respectively. This is not in favour of the recommendation by Mata-Alvarez (2003) where alkalinity is preferred over pH when monitoring an anaerobic reactor. Mata-Alvarez (2003 p 157) argued that alkalinity variation is faster than the pH variation. As shown by Figure 5.21, when pH variations are observed, the alkalinity of the system, and thus buffer capacity, was already lost.

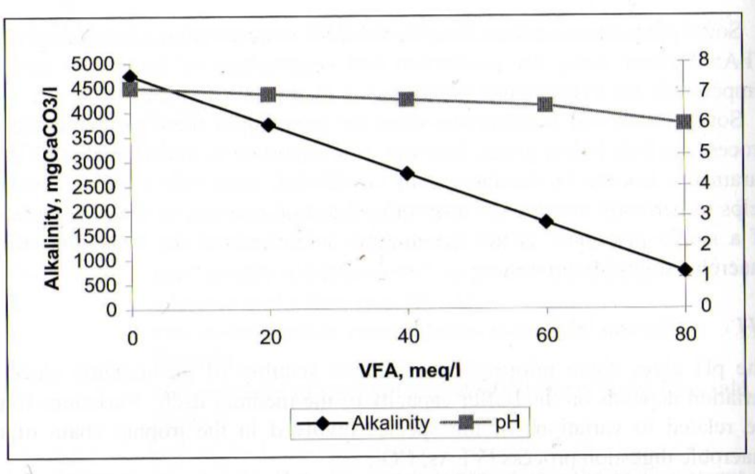


Figure 5.21 pH and alkalinity profile versus VFA concentration in the reaction medium (source: Mata Alvarez, 2003)

As per Mata-Alvarez (2003), this research also observed a faster variation with alkalinity compared to pH (refer Section 5.3.1 and Figure 5.1). Having said that, alkalinity data was more irregular than pH was in this research (refer Section 5.3.2), prompting the preference to the latter due to greater confidence in the data. The result of Figure 5.1 suggests that a high alkalinity concentration can be misleading and does not necessarily mean that a reactor is operating under a stable condition. The meat based reactors had alkalinity as high as 25000 mg/l. Yet, the reactors were less than healthy as seen with TVFAs above 10100 mg/l and pH average of 6.5. The reactor alkalinity could also be substrate dependent. Because protein is a major constituent in meat, it is not surprising that ammonia content from the meat reactors are high compared to the food waste digesters. The high ammonia reaching 4900 mg/l may have led to the high alkalinity in the meat reactors.

Another factor was, despite the occasional buffering with phosphate buffers to try restoring the reactor condition, the alkalinity of the meat and meat and sawdust reactors did not improve. This was not expected as the notion was alkalinity would increase with the added buffer. Either alkalinity was not sensitive or as pointed out by Chynoweth and Isaacson (1987), attempts to increase pH to recover an unstable digester do not necessarily ensure increase in alkalinity.

In this research, the pH and methane percentage were generally sharp at signifying the reactor is going acidic and methane production is ceasing. These parameters are preferred as both can be measured in real time by use of portable devices. Samples are analysed as is, cutting preparation time plus the results can be obtained instantly. Many researchers (Wang et al. 2002; and Cecchi, et al., 1991) have also found these parameters suitable to monitor digestion progress. Bouallugui et al. (2003, 2004) agreed pH as a sensitive parameter to determine reactor stability. Cecchi et al. (1991) reported CH_4 percentage and pH as the only two parameter which are able to describe the acid-base state of a digester.

5.4.4 Factors and solutions for process imbalance

Washout, presence of inhibitors e.g. due to excessive ammonia, and sudden temperature drop were found to cause imbalance in the anaerobic digesters. When ammonia is in high concentrations, for example pig manure in the work by Angelidaki and Ellegaard (2005), the anaerobic reactor is more stressed and losing more potential biogas production with digester effluent (Angelidaki and Ellegaard, 2005).

For all the factors, the symptoms of imbalanced process includes drop in methane concentration and pH, followed by a rise in VFA and SCOD. A healthy digestion would otherwise produce more than 50% CH_4 under neutral reactor pH environment, with VFA and SCOD concentrations below 1000 and 10000 mg/l, respectively. This is in agreement to the studies of Angelidaki and Ellegaard (2005) and Kaparaju and Angelidaki (2008) who saw high and fluctuating VFA linked to temperature instability. Mesophilic processes operate well in the range of 30-40°C and a variation of some 2-3°C in temperature can give rise to a change of the system. This is because different temperature ranges determine totally different bacterial populations (Mata-Alvarez, 2003).

Washout occurs when a greater number of microorganism leaving the system daily in the effluent (Solera et. al., 2001a). The reactor used in this research was a simple one stage semi-continuous stirred tank reactor. Due to the simplicity of the reactor design and lack of biomass retainment, loss of microorganisms with the digester effluent was a potential issue. Especially so as reactor feeding and wasting were done directly after mixing. Because of mixing, the reactor content gets re-suspend and the effluent contains the same concentrations of bacteria as the digester itself (Chynoweth and Isaacson, 1987). Hence, wasting some of the reactor content directly after mixing means it is more likely that some of the bacteria are dispensed from the reactor at the same time. After some time, repetitive export of bacteria not balanced by the bacteria population from the import of fresh feed materials can be detrimental to the reactor environment. The slower-growing methanogenic bacteria will wash out of the reactor more rapidly than the faster-growing, acid-producing bacteria, causing a microbiological imbalance, accumulation of high levels of volatile acids, and eventual destabilization of the system (Chynoweth and Isaacson, 1987). Not only were the methanogens being washed out from the reactor, some of the reactor micronutrients are likely to have been depleted from the digester in the same way. Inocula at reactor start-up often is rich in micronutrients compared to the substrate, therefore washout problem becomes worse with time. Micronutrients are like food to the bacteria and without them, the methanogens could starve and stop multiplying.

Restoring digester stability requires understanding and identifying the cause of destabilization. The corrective measures can then be implemented. This research has seen the benefits of utilizing effluent from another reactor, trace elements addition and reducing feeding during events of washout and temperature upsets. One of the key findings of this Chapter is the need for operating at least 2 reactors concomitantly. The FW2.6 which suffered from washout was able to bounce back from a deteriorating situation after 57 days of utilising effluent from another reactor and 22 days of adding trace elements. It took less than 2 weeks for the FW5 and FW3 to regain its healthy performance by reducing reactor feeding and adding effluent from another reactor following a temperature room malfunction. Because process failure was associated with an increase in VFA concentration, positive effect was reported from temporary stopping of reactor feeding (Wang and Banks., 2000). Canovas-Diaz and Howell (1988) following starvation over 2 days, saw VFA decrease to less than 200 mg/l. The OLR was then gradually raised and reactors regained normal performance within 2 days.

In spite of the suggestions by several researchers to dilute the reactor content in the event of ammonia inhibition, this research did not see any benefit of such a treatment as has been discussed in Section 5.3.3. Reactor dilution relies on the decrease of N concentration (Mata-Alvarez, 2003) and pH regulation to reduce the effect of ammonia inhibition (Kayhanian and Tchnobonglous, 1993). Running digesters with high ammonia levels at relatively long HRT in order to avoid accumulation of VFA and retain stable process has been suggested as a treatment method (Schurer and Norberg, 2007). This research ran the meat reactor at 166 days HRT, however, ammonia (at 5000 mg/l) still caused reactor inhibition. As stated by Chynoweth and Isaacson, 1987), microbial cells might have washed out before acclimation occurred. Adding trace element supplementation possibly will help resolve this issue by encouraging better microbial growth.

5.5 CONCLUSION

To start and operate a healthy continuous reactor for the first time is not an easy task. Without the appropriate guidelines and steps to refer upon, inexperienced operators might blindly run a reactor under 'inhibited steady state' conditions. This is a condition where the reactor is running stably but at a lower methane yields.

Based on the findings of this chapter the following steps and thresholds values are concluded to be reliable in ensuring a successful digestion. The guideline was drawn through experience digesting several substrates; meat, meat and sawdust, cabbage and food waste under mesophilic temperature in a continuous reactor. Conclusions are provided in the form of advice to experimenters operating bench-scale anaerobic reactors.

1) How to start a reactor?

The first step is to establish the suitable OLR and HRT specific to the substrate. These two parameters were shown to influence the stability and performance of the reactors. A HRT of about 20 – 30 days are most common for a simple one-stage continuous system. The OLR can be determined by conducting batch scale tests first and then scaled-up using a factor (this would be discussed in Chapter 6). Conducting literature review on similar substrate and operating system (temperature, continuous, one stage) is also recommended. This is so that the reality for digesting the substrate can be realised such as the lowest, average and highest OLR achievable. Comparison on expected methane yields and problems can be also be foreseen. After establishing the OLR, the HRT can be modified as required by diluting the feedstock or adjusting the feed/waste frequency. HRT for a non-recycled reactor system is obtained by dividing the reactor working volume with the amount of feeding.

2) How long is start-up and how to know start-up is successful?

This research finds that a large inoculum volume followed by steps to counter acidification effects helps to assure a successful start-up. The following start-up procedure is recommended:

(i) Reactors are best filled only with seed sludge preferably at the start. Feeding should start after allowing the seed to adapt to the incubation temperature for 7 days minimum.

(ii) When feeding, buffer and nutrient should also be added until a significant methane percentage has build-up. This is generally about 50% CH_4 within 9 days. Buffering should then stop to allow the reactors to self regulate.

(iii) Within 5 days after starting, a healthy reactor would proceed to a steady state condition as the initial acid production has passed. On the other hand, a troubled reactor would generally fail even though buffer was provided within this time period. Therefore, 1-2 weeks maximum should be used a guideline to decide if the reactor ought to proceed or not. The general rule of a potentially successful system is when after 5 days of start-up, the reactor emitted 50% CH_4 with roughly neutral pH.

3) After start-up, how do you maintain a healthy the reactor?

To maintain a reactor, the approach is to be aware of the reactor system stability. This can be done through a quick check. The rule is a stable reactor would have a low VFA with a high methane percentage, pH and alkalinity. An unstable reactor would instead depict a high VFA concentration followed by low pH, methane percentage and alkalinity. If any of the above parameters does not follow this rule, the ‘peculiar’ parameter should be checked for measurement error. Ammonia in a healthy reactor should be low; enough to provide necessary nutrients but not excessive that it would become toxic. The question then lies to how high is high and how low is low for a parameter, in order to confirm the reactor stability. Checking against the threshold values in Table 5.4 would suggest the stability of the reactor. Another means to maintain a healthy operation is to ensure that sufficient bacterial or micronutrient is retained within the reactor. This can be achieved by adding trace elements to the reactor especially following a long reactor operation (e.g. 90 days). The extra nutrients would help compensate for any that was depleted from the system as well as stimulate bacterial growth.

4) If the reactor is stressed, what should we do?

When a reactor is stressed, this is usually linked to an imbalance between process of hydrolysis/acidogenesis and methanogenesis. This generally means the methanogenic population is inadequate to prevent the build-up of the organic acids. The recovery methods suggested here involve ways to calm down the acid accumulation and at the same time allow the methanogenic population to double and become stronger. Methanogenic bacteria are slow growing microorganisms with reported doubling times of 3-6 days. If the reactor stress was due to washout or sudden temperature drop, a temporary cessation of feeding for a day for one week might work. If not, the troubled reactor would in time turnaround after extra anaerobic bacteria e.g. transferring effluent from another reactor or trace elements are provided. Recovery for a reactor operating at low loading rate reactor is faster than a higher loading rate one. Reactor dilution with deoxygenated water following ammonia inhibition was less beneficial considering the time needed to build-up the methane production. If possible, starting a new reactor is recommended instead as it is faster (1-2 weeks).

CHAPTER 6

KINETICS OF REACTION

6.1 INTRODUCTION

The anaerobic treatment plant is generally designed and operated on the basis of empirically derived information. This usually involves data on biogas yield and composition along with chemical oxygen demand (COD) and volatile solids (VS) reduction gained from laboratory tests. As observed by Caffaz et al. (2007) the gas production rate (GPR) data of the pilot plant agreed well with the GPR of the laboratory batch test of the pilot plant digested sludge. Brunn et al. (2007) instead observed differences in the measured parameters (VS, total organic carbon (TOC), ammonia (NH_3-N), and organic acids) between the two scales as well as the gas yields. The small sample amount that was used in the lab tests may have been a contributing factor because they may have been less representative of the average of the substrates going through the industrial plant. It seems here that experimental data if used without careful consideration may cause digesters to be poorly designed and subject to unpredictable failure in performance.

The use of kinetics that considers the underlying metabolic and biological principles governing the whole process, should lead to more economic design and predictable stable performance. Kinetic studies offer deeper insight into the mechanisms of hydrolysis attack, effects of different pre-treatments and prediction of bioconversion and effluent quality, which can be overlooked when designing a full-scale plant. It is the focus of this chapter to look at the suitability of laboratory experiments towards predicting the performance of an industrial plant through knowledge of its kinetics. Batch systems, namely the developed tube and bottle, are studied to assess their ability to exemplify the anaerobic digestion of a similar sample in a continuous reactor system. Bench or pilot-scale continuous reactor (CSTR) systems are a closer match to a full-scale continuous treatment project. First order kinetics will be applied, where the methane yield and first-order rate constants are compared and relationships developed (if any) between the various reactor systems. The suitability of first order kinetics for achieving this purpose will also be discussed.

6.2 METHODOLOGY

6.2.1 Feedstock and Inoculum

The feedstock for the experiments was mainly food waste and rice prepared according to the Methodology Chapter. The inoculum was digested sewage sludge (DSS) sourced from the Christchurch Wastewater Treatment Plant (WWTP) pre-digested for 7 days before use at 35°C. The amount of substrate and seed added to each reactor is detailed below.

6.2.2 Experimental Procedure

The aim here is to compare the different reactor systems; and investigate the corresponding kinetics. Therefore, the operating conditions between them are kept at similar organic loading rates between the batch reactors but may differ with the continuous reactor. Food waste was digested in all reactor systems while rice only in the batch systems. Following is their description:

6.2.2.1 Food waste

For the batch system, the developed tube and bottle are stored for more than 20 days in an incubator at 35°C and operated at an organic loading rate of 18.8 g VS/L. d. This means for the tubes, 120 g food waste is added to 1.5 L DSS while for the bottle, 16 g food waste is added to 200 ml of DSS. The tubes are operated as described in Chapter 4. Approximately thirteen tubes are tested; Test 1, a set of triplicate in Test 2 and Test 4, while six replicates are analysed in Test 3. These tests were performed at different times. Exactly 10 bottles were analysed, 5 replicates each at two different times. These were labelled as Test 5 and Test 6.

For the continuous system, two 30 L stainless steel digesters as described in Chapter 3 were employed. These digesters were loaded at 3.0 and 1.5 g VS/L. d organic loading rate. The hydraulic retention time (HRT) is 30 days. About 50 ml of phosphate buffers (100 mg l-1) were added with the feedstock daily until Day 7 so as to counter any acidification effect in the digester. A trace element solution (5 ml) as per Methodology Section in Chapter 4 is also supplemented to avoid occurrence of nutrient deficiency due to washout of the initial nutrients in the microbial seed. Other operating conditions, feeding/wasting and sampling procedure is according to the methods in Chapter 3.

6.2.2.2 Rice

Rice (Sunrise Basmathi) was tested at 11 g VS/L. d organic loading rate (OLR) for both tube and bottle batch tests for more than 20 days at 35°C. Approximately 50 g (w/w) rice was introduced in the tubes while 7 g (w/w) rice was allowed in the bottles. This is followed by DSS as seed filled to 1.5 L for the former and 200 ml for the latter. Four tubes are tested while 11 bottles are analysed consisting of one replicate and 5 replicates tested at different times. These were labelled as Test 10a, Test 8a-e and Test 9a-e.

6.2.3 Analyses

Analyses for tubes and CSTR are similar to the description in the Methodology Chapter. For the Schott bottles, the biogas collected in the 5 L Tedlar bags are emptied daily for the first 5 days and with a period of 4 days thereafter. The biogas volume collected is measured by squeezing the bags into a water displacement cylinder. The biogas composition is determined via gas chromatography (GC HP5890). Solids analysis, volatile fatty acid (VFA), ammonia, COD and pH of the effluent were taken at the beginning and after the incubation period according to the methods described in Chapter 3.

6.2.4 Kinetics

As mentioned earlier, first order kinetics was considered to predict the performance of various reactors and validated against the experimental data. The first order model is as Equation 6.1 where S is the volatile solids concentration and k (d^{-1}) is the first order rate coefficient (Vavilin et al., 2007):

$$dS/dt = -kS \qquad \text{Equation 6.1}$$

For a batch system, cumulative methane production data was analysed using first order kinetics as given in Equation 6.2 (Prashanth et al., 2006):

$$B = B_0*(1-e^{-k(t-tlag)}) \qquad \text{Equation 6.2}$$

where B is cumulative methane at time t (L CH_4/g VS); B0 is ultimate methane yield (L CH_4/g VS), k is first order rate constant (d^{-1}), t is time of incubation (d) and t lag (d). The best fit curves with respect to the experimental data were obtained using non-linear regression with the function Solver in Microsoft Excel. This function estimates values of model parameters by minimizing the sum of squared differences (SSR) between observed and predicted values:

$$\mathrm{SSR} = \sum_{i=1}^{n} (\mathrm{y\ calc} - \mathrm{y\ exp})_{i}^{2}$$

Under steady state conditions for continuous reactors, the first order kinetics can be described as per equation 6.3 (Mata Alvarez, 2003):

$$B = B0\ (k.\ HRT/(1 + k.\ HRT)) \qquad \text{Equation 6.3}$$

where B is the specific methane production (L CH_4/g VS), k is the first-order rate constant (d^{-1}) and HRT is the hydraulic retention time (d). In this research, the B value is obtained from the continuous system, while the B0 are from the tube batch degradation tests. These values are then substituted into the above equation to obtain the hydrolysis rate constant for the continuous reactors.

6.3 RESULTS

6.3.1 Kinetics of individual reactor systems

6.3.1.1 Food waste digestion

a) Batch tubes

Table 6.1 shows the first order kinetic parameters that were estimated for a food waste digestion in the tubes. The estimated ultimate methane yield (B0) varied greatly, and the results in Table 6.1 were sorted in ascending order of the B0. Based on the SSR values, it can be said that the parameters was estimated reasonably accurately using the non-linear regression method. This is because the SSR values were satisfactorily very small (the highest being 0.0086). The data especially that of the ultimate methane yield (B0) and the first order rate constant (k), are then transferred to a histogram (refer Figure 6.1 and 6.2) to better visualize the predicted parameters. A worked example of first order parameter estimation on data of Test 1a in Table 6.1 is given in Appendix 6-a.

Earlier in Chapter 4, the theoretical value for methane yield of food waste was established to be 0.45 – 0.50 L CH_4/g VS. Taking a B0 of 0.45 L CH_4/g VS in Figure 6.1, which is represented by the dotted line, the histogram shows there is a 54% percent probability (represented by the shaded area) that any one sample will lie within $\pm$ 0.1 L CH_4/g VS of the maximum. This means there is a moderate possibility (up to 35 percent) that a sample could fall outside of the $\pm$ 0.1 L CH_4/g VS band of the average. As seen in the histogram (Figure 6.1) samples are more likely to give a limited methane yield (lower than 0.35 L CH_4/g VS) rather than excessive yields (over 0.55 L CH_4/g VS) as a sign of a troubled process.

Focusing on those tests where the ultimate methane yield was between 0.35 to 0.56 L CH_4/g VS, the corresponding average first order rate constant was established to be 0.20 d^{-1}. The histogram (refer Figure 6.2) shows that the likelihood that a sample will fall within 0.12–0.28 d^{-1} range (which is $\pm$ 0.08 d-1 of the average) is 63% (represented by the shaded area).

Table 6.1 Kinetics of 18.8 OLR food waste tested in tubes after 20 days incubation

Test	B0 (L CH4/ g VS)	k (d-1)	t lag (d)	SSR	Comment
4b	0.1455	0.1448	0.0000	0.0003	*Unacceptable values*
3a	0.1705	0.2423	0.3483	0.0009	
3b	0.2308	0.4228	1.0471	0.0007	
3c	0.2519	0.2608	1.1991	0.0015	
4c	0.3136	0.0386	1.0415	0.0009	
Average	*0.2225*	*0.2219*			
Stdev	*0.07*	*0.14*			
C.V. (%)	*30.0*	*64.5*			
3d	0.3503	0.1917	0.6519	0.0007	*Acceptable values*
3e	0.3699	0.2923	0.6256	0.0008	
3f	0.3948	0.2804	0.6305	0.0010	
2b	0.4134	0.1566	0.6134	0.0039	
4a	0.4312	0.1170	0.0000	0.0049	
1a	0.4434	0.4745	0.6320	0.0010	
2c	0.5129	0.1481	0.7155	0.0086	
2a	0.5595	0.1318	0.4883	0.0035	
Average	*0.4344*	*0.2241*			
Stdev	*0.07*	*0.12*			
C.V. (%)	*16.3*	*53.8*			
Average	*0.3529*	*0.2232*			*All 13 samples*
Stdev	*0.13*	*0.12*			
C.V. (%)	*35.8*	*55.4*			

*The tests having the same number denote the number of replicates tested on the same run. SSR: sum of squared difference. Stdev: standard deviation. C.V.: coefficient of variation

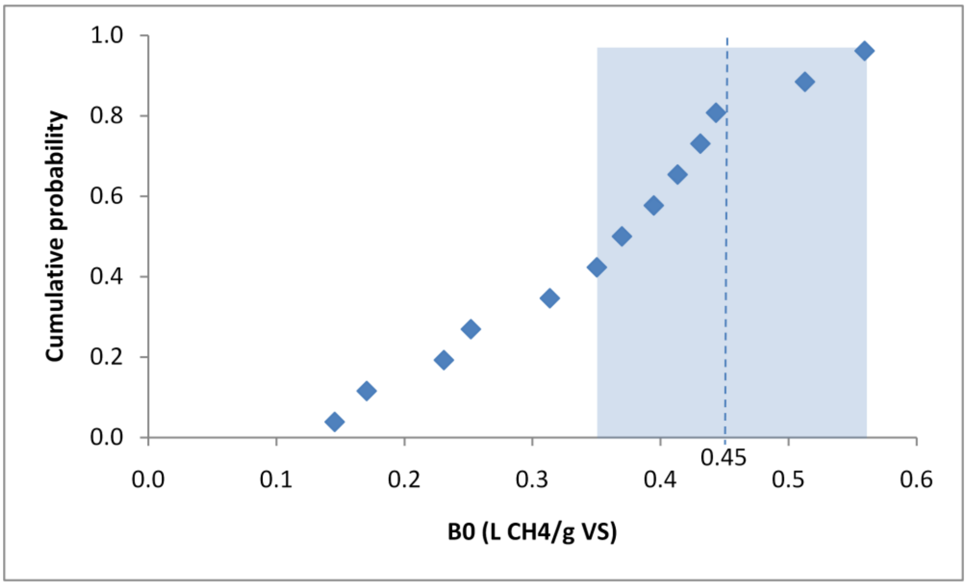


Figure 6.1 Cumulative probability graph of the estimated B0 values corresponding to Table 6.1

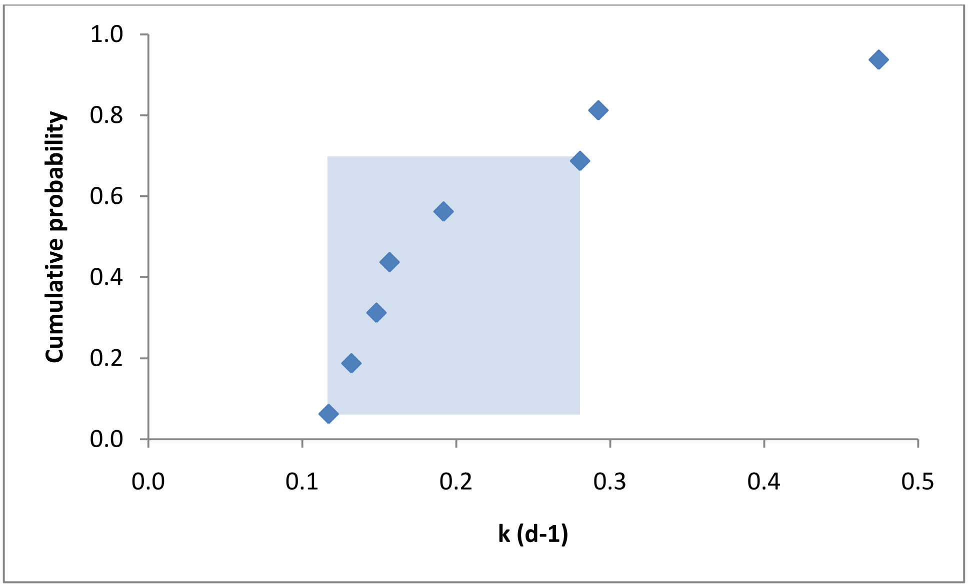


Figure 6.2 Cumulative probability of the estimated k values corresponding to B0 of 0.35 – 0.56 L CH_4/g VS

No strong correlation however can be seen between these estimated B0 and k for these data as shown by Figure 6.3. This was also true for the relationship between these two parameters and t lag (refer Figure 6.4). Figure 6.5 indicates that excluding t lag in the first order equation (refer Equation 6.3) can overestimate the B0 value while underestimating the k value. This effect is more pronounced for the rate constant achieving up to 14% difference. However, except for the 4c data, the ultimate methane yield does not differ much, varying at just 3%. For all 13 replicates, the removal of lag registered a C.V of 36.6 % for B0 and a C.V of 48.2 % for k. The coefficient of variation of B0 and k for the 8 replicates that were 'within range' was 16.8 % and 45.4 %, respectively, after removing the lag. Compared to the C.V. values of B0 and k where t lag was included (C.V. B0=35.8%, k=55.4% for all 13 replicates; C.V B0=16.3%, k=53.8% for the 8 acceptable values; refer Table 6.1), it is clear the removal of t lag from the first order equation did not cause a significant reduction in the coefficient of variation. Therefore it can be suggested that t lag can be included or excluded from the first order equation without much affect on the estimation of the first order parameters. It may be more suitable to remove the t lag term when extrapolating batch test values of k to larger-scale, continuous tests. Further work on effect of t lag term for reactor scale up would be beneficial.

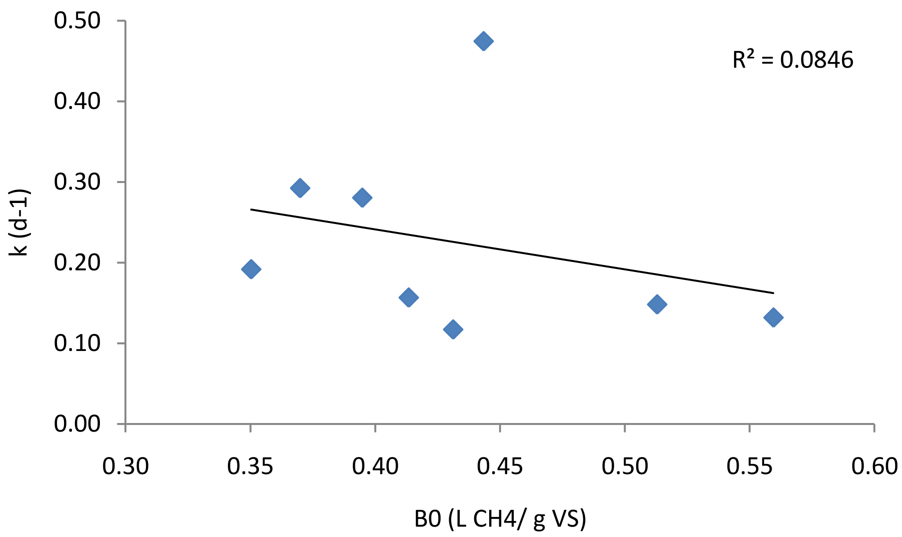


Figure 6.3 The relationship between predicted ultimate methane yield (B0) and first order rate constant (k) for those results with estimated B0 between 0.35 – 0.56.

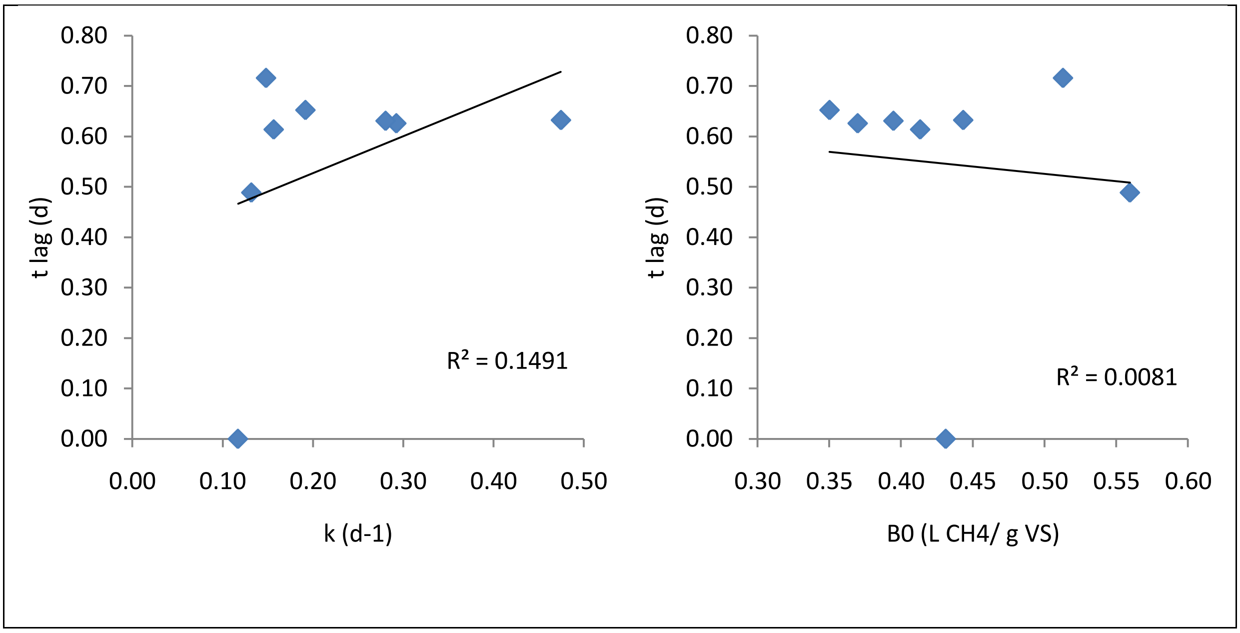


Figure 6.4 The relationship between predicted t lag and ultimate methane yield (B0) and first order rate constant (k) for those results with estimated B0 of 0.35 – 0.56 L CH4/g VS

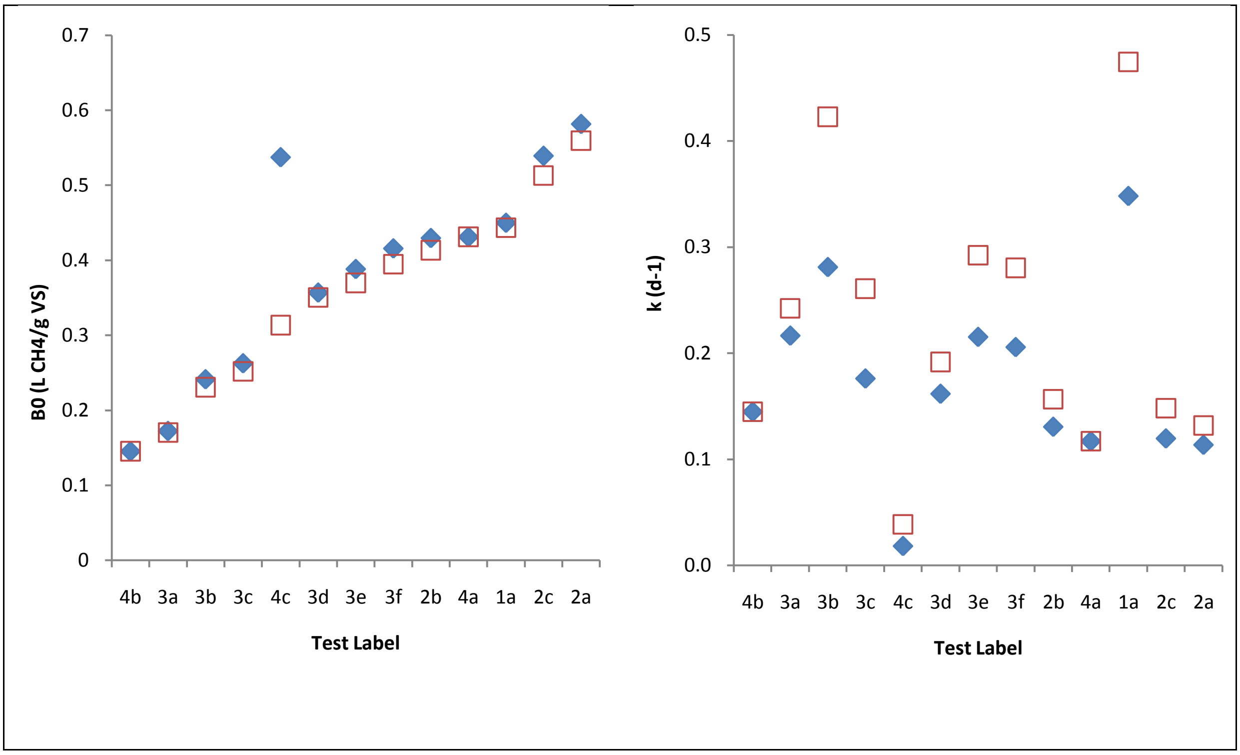


Figure 6.5 Effect of the estimated B0 and k values if the t lag is assumed zero for all estimated B0 values. The square symbol represents the condition if t lag is added to the first order equation, while the diamond symbol if t lag was not

By graphing the experimental data to its predicted behaviour, it was hard to detect any possible factors that could limit its performance. This is because under both conditions where tubes are performing within range[1] (refer Figure 6.6) and outside of range[2] (refer Figure 6.7), the fit between experimental and predicted was reasonably accurate. The SSR values for the data in Figure 6.6 is between 0.0007 to 0.0086, while for Figure 6.7 the SSR ranges from 0.0003 to 0.0015. Part of the problem of less consistency with the food waste could probably be attributed to sample-to-sample variations exhibiting varied sample degradation. Both Figures (6.6 and 6.7) seem to suggest that when the SSR values are considerably small (<0.009), a good fit between expected and experimental behaviour can generally be expected. To help explain why some samples fell outside of the acceptable ranges for B0 and k, the experimental data used in the first order modelling was looked at as in Table 6.2.

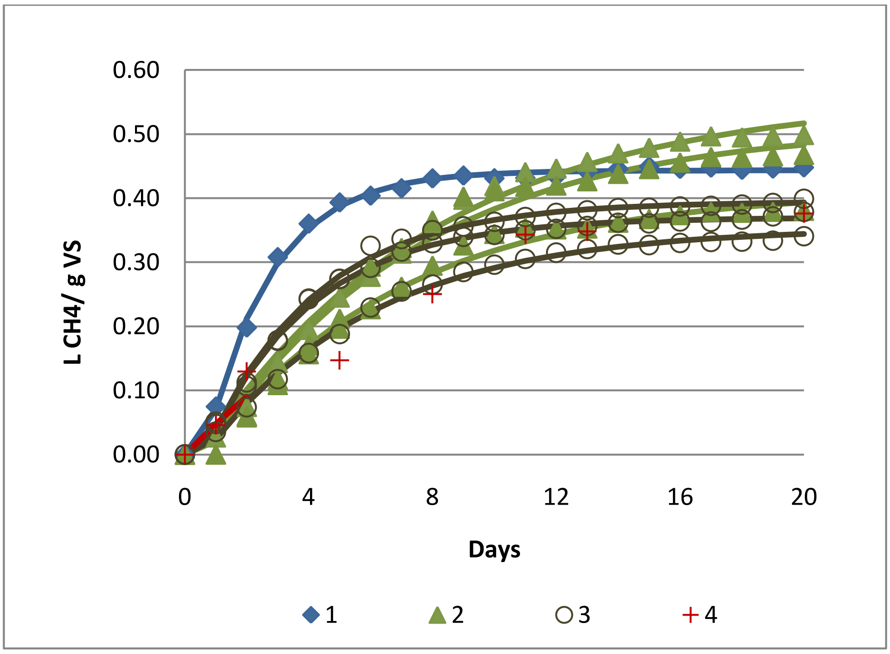


Figure 6.6 Methane yield of 18.8 OLR food waste matching to Table 6.1 with kinetic parameters inside ‘acceptable’ range. Symbols represent yields from experiments while the corresponding lines are yields fitted using first order kinetics.

[1] Data where B0 is between 0.35 – 0.56 L CH_4/ g VS
[2] Data where B0 is not between 0.35 – 0.56 L CH_4/ g VS

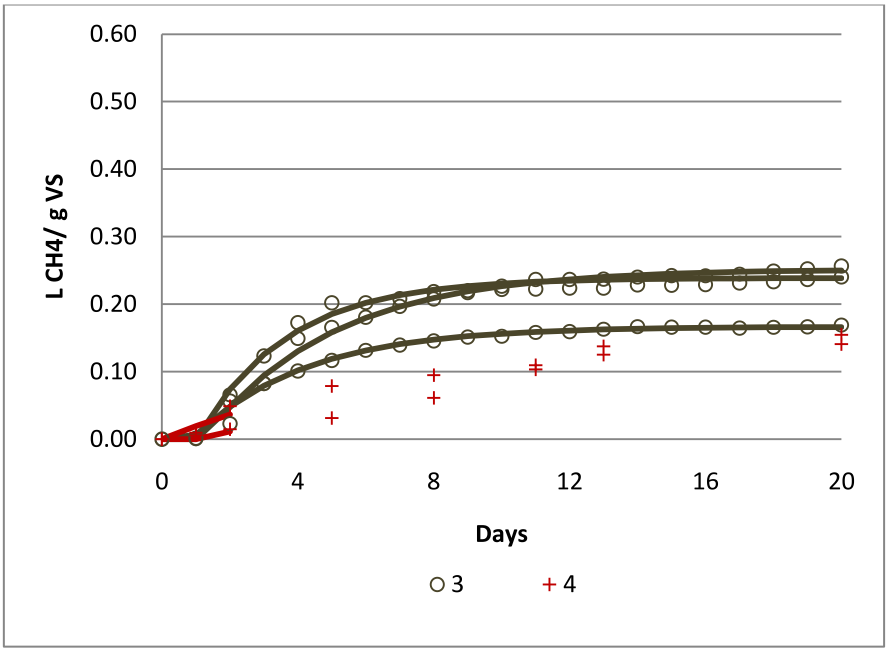


Figure 6.7 Methane yield of 18.8 OLR food waste matching to Table 6.1 with kinetic parameters out of 'acceptable' range. Symbols represent yields from experiments while the corresponding lines are yields fitted using first order kinetics

No concrete explanation can be offered to as why methane production was limiting for some of the tests even though the reactor operating and substrate conditions were the same. The amount of organic matter reduced and the volume of methane produced for all the Test conditions appears to be balanced. When the reactor effluent was tested, no apparent pattern could be seen between the different Tests (1a, 3a - 3f) and their chemical composition (refer Table 6.3). The pH was neutral around 7.0 to 7.5 (Table 6.3) for all the Tests (1a, 2a – 2c, 3a – 3f and 4a – 4c). The ammonia levels ranged from 1100 to 1600 mg/l for Test 1a and Test 3a-3f, while the alkalinity was around 4500 to 6700 mg/l $CaCO_3$. For most of the tests, the soluble COD was fairly similar between 1300 to 1700 mg/l except for 3a and 3b which had a soluble COD (SCOD) of 0 and 325 mg/l, respectively. Referring to the findings of Chapter 5, the SCOD and alkalinity concentration is not that of a stressed reactor. A stressed reactor would normally contain around 2000 – 5000 mg/l SCOD and more than 20000 mg/l alkalinity. Ammonia on the other hand is quite high where levels between 1000 – 2000 mg/l have been reported to be indicative of an unstable system. When compared with their pH values, no distinct pattern can be seen to suggest the inhibiting effect of ammonia and pH combination. Tests which had ammonia above 1300 mg/l and pH above 7.4, recorded a varied methane yield, from as low as 0.23 L CH_4/g VS to as high as 0.44 L CH_4/g VS. It has to be remembered that out of the Tests reported in Table 6.3, Tests 3a, 3b and 3c, fell outside of the ‘acceptable’ range for B0 (0.35 – 0.56 L CH4/g VS) and k (0.12-0.28 d-1). However, these Tests (3a – 3c) had ammonia, SCOD and alkalinity concentrations which are no different from the others (Test 1a, 3d, 3e and 3f). The total volatile fatty acid (TVFA) pattern also is considered representative of a stable process as the high VFA production at Day 2 of 2635 mg/l, drop further to 1059 mg/l at Day 5 before barely appearing from Day 13 onwards. This signifies that the acid burst in the early digestion is later taken for methane production and an accumulation of VFA is avoided.

Table 6.2 The pH, experimental methane yield and volatile solids reduction of 18.8 OLR food waste digested in the tubes

Test	Measured Yield[a]	VS reduction	Total measured CH_4[b]	CH_4[c] not yet produced	Theoretical[d] CH_4 not measured, %	pH
	L CH_4/g VS	%	ml	ml		
4b	0.1410	90	2848	323	69	7.4
3a	0.1690	92	3423	302	62	7.3
3b	0.2440	100	4945	0	46	7.4
3c	0.2580	n/a	5215	n/a	43	7.5
4c	0.1550	97	3130	110	66	7.4
3d	0.3600	95	7283	408	20	7.4
3e	0.3930	93	7957	608	13	7.0
3f	0.4290	93	8692	674	5	7.4
2b	0.3800	91	7697	733	15	7.4
4a	0.3760	96	7612	325	16	7.5
1a	0.4480	70	9063	3487	0	7.5
2c	0.4680	90	9471	1064	**6**	7.4
2a	0.4990	93	10092	736	**0**	7.4

Note: a: methane per g VS as measured in the 20 day experiments; b: total volume of methane measured in the experiments; c: Volume of methane not yet produced in 20 days (details of calculation is given in Appendix 6b); The theoretical methane volume for food waste at 0.45 L CH_4/g VS is 9108 ml, while at 0.50 L CH_4/g VS is 10120 ml. d: the theoretical methane volume – b

Table 6.3 The ammonia, soluble COD, alkalinity and volatile fatty acids of 18.8 OLR food waste digested in the tubes.

Test	SNH_3-N (mg/l)	SCOD (mg/l)	Alkalinity (mg/l)	TVFA[a] (mg/l)	pH	Day[b]
1a	1650	1450	5700	n/a	7.5	20
3a	1105	0	6700	n/a	7.3	20
3b	1343	325	5700	< 696	7.4	40
3c	1160	1550	4800	n/a	7.5	27
3d	1368	1325	4500	< 696	7.4	62
3e	1220	1750	6000	< 696	7.0	56
3f	1313	1725	4800	< 696	7.4	62

Notes: [a] Similar tubes that were tested at Day 2 had 2635 mg/l TVFA, at Day 5 = 1059 mg/l and at Day 13 had VFA < 696 mg/l. [b] Indicates the time reactor incubation is completed and samples taken for the ammonia, SCOD, alkalinity and VFA tests. Highlighted cells represent tests that are outside of acceptable range for B0 and k.

Figure 6.8 is a contour plot showing the joint uncertainty for the estimated values of k and B0 for all reactors under ‘acceptable’ conditions. Acceptable conditions are when k is between 0.12-0.28 d^{-1} and B0 is between 0.35-0.56 L CH_4/g VS. A wide gap is evident in the contour between SSR 0.000 to 0.003. Here, there is a widespread region of potential B0 and k values; the lowest B0 was at 0.15 L CH_4/g VS with k of 0.15 d^{-1}. The highest B0 was 0.44 L CH_4/g VS while the k was 0.47 d^{-1}.

Focusing on this B0 and k envelope, generally it looks like as the reaction rate constants are higher, the ultimate methane yield becomes less. Provided that the methanogenesis proceeds at a constant of 0.12 to 0.28 d^{-1}, it can be logically deduced that 0.35-0.45 L CH_4/g VS can be produced under ‘real’ digester conditions. If all conditions are at an optimum, a higher methane yield between 0.40 to 0.50 L CH_4/g VS, closer to the theoretical values can be achieved, though perhaps at a lower k.

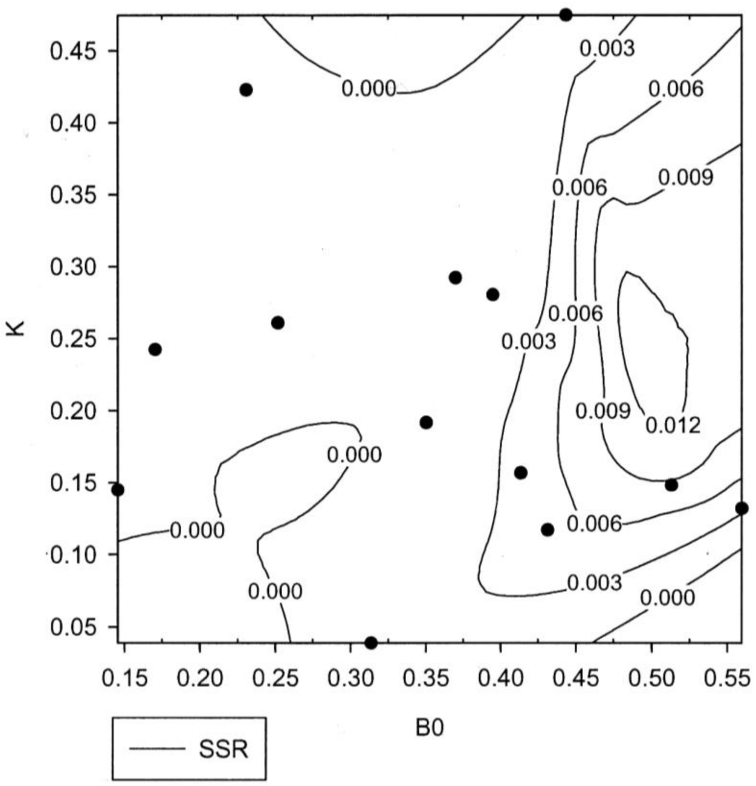


Figure 6.8 The distribution of B0, k and the sum of squared residual (SSR) for food waste at 18.8 OLR in the batch tubes. The dots are the data (obtained from both 'acceptable' and 'unacceptable' range) in the experiments.

b) Batch bottles

Table 6.4 represents the first order kinetic parameters estimated by Equation 6.2 for food waste in 250 ml Schott bottles. Apart from the scaled down effect of sample size (7 g w/w) and seed volume (200 ml), all other operating conditions were similar to the conditions for the tubes.

Table 6.4 Kinetics of 18.8 OLR of food waste in 250 ml bottles after 20 days

Test	B0 (L CH_4/g VS)	k (d^{-1})	t lag (d)	SSR
5d	0.0088	0.2925	0.8941	0.0000
6b	0.0240	0.3931	0.2011	0.0000
6c	0.0308	0.2125	0.2491	0.0000
6e	0.0525	0.4175	0.0000	0.0000
6a	0.0677	0.4282	0.1175	0.0001
5e	0.0842	0.1935	0.5341	0.0002
6d	0.1428	0.2684	0.2784	0.0010
5a	0.1722	0.3053	1.0917	0.0007
5b	0.2210	0.3097	0.2093	0.0017
5c	0.2500	0.3097	0.2093	0.0024

The highest SSR was just 0.002 with most data estimated at SSR of 0.000. Unfortunately, despite the high sensitivity in parameter estimation, the achievable B0 was only 0.25 L CH_4/g VS which is considerably short of the theoretical maximum value found in the tubes. In fact as shown by the cumulative probability graph in Figure 6.9, 55% is likely to produce less than 0.1 L CH_4/g VS. On the other hand, k was larger ranging from 0.22 to 0.43 d^{-1}, when compared to the batch tubes. As shown by the histogram in Figure 6.10 about 50% of replicates will fall within the 0.08 d-1 envelope from an average of 0.30 d^{-1}. Another 50% is predicted to fall outside of this range. The predicted B0, k and t lag appear to be independent of each other. As shown by Figure 6.11 and 6.12 no relationship can be drawn between them. Figure 6.13 illustrates the joint uncertainty of the first order rate constant, ultimate methane yield and SSR in a contour plot form. As can be observed the predicted values seem to be all over the place. No discerning behaviour especially between the rate constant and methane yield can be deduced.

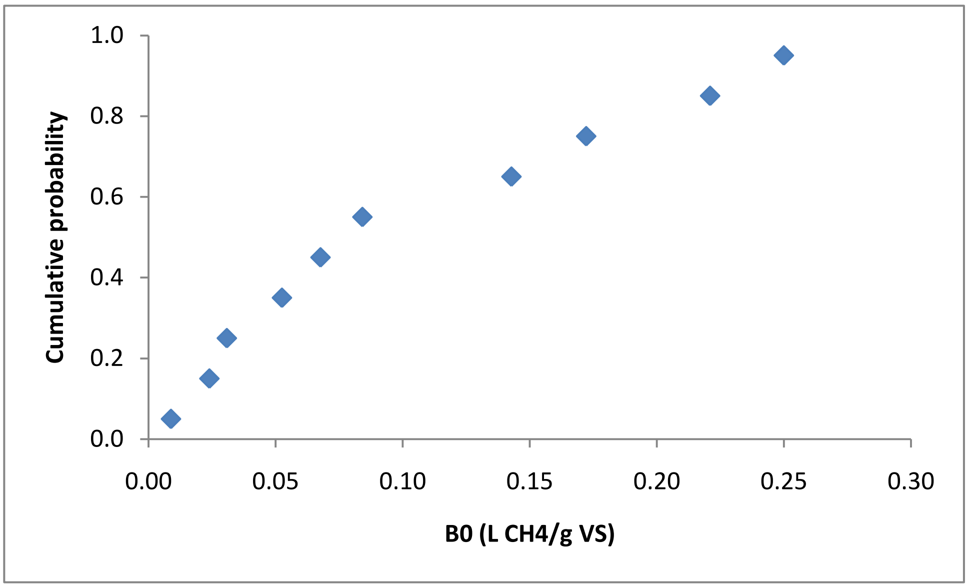


Figure 6.9 Cumulative probability graph of methane yield for 18.8 OLR food waste measured in the bottle after 20 days

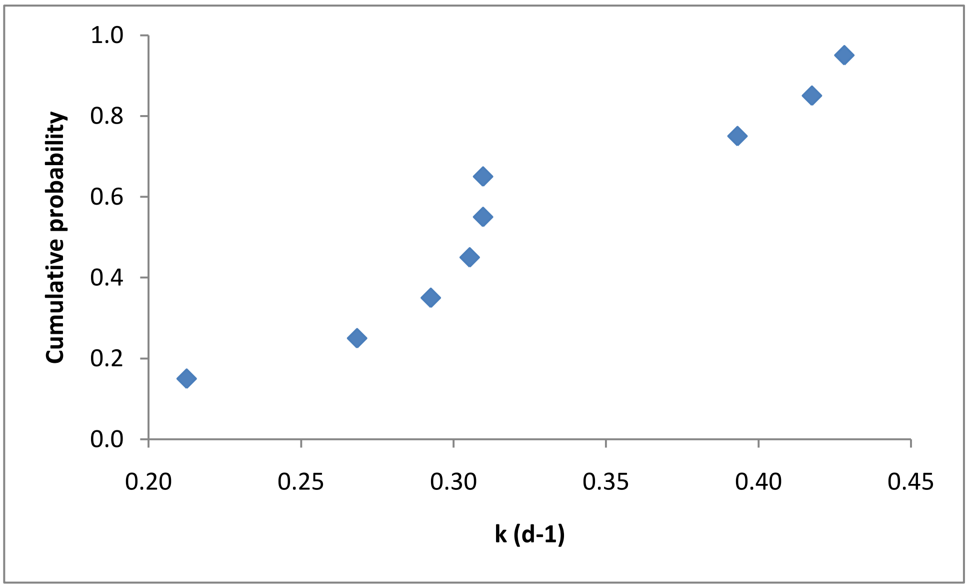


Figure 6.10 Cumulative probability graph of rate constant for 18.8 OLR food waste measured in the bottle after 20 days

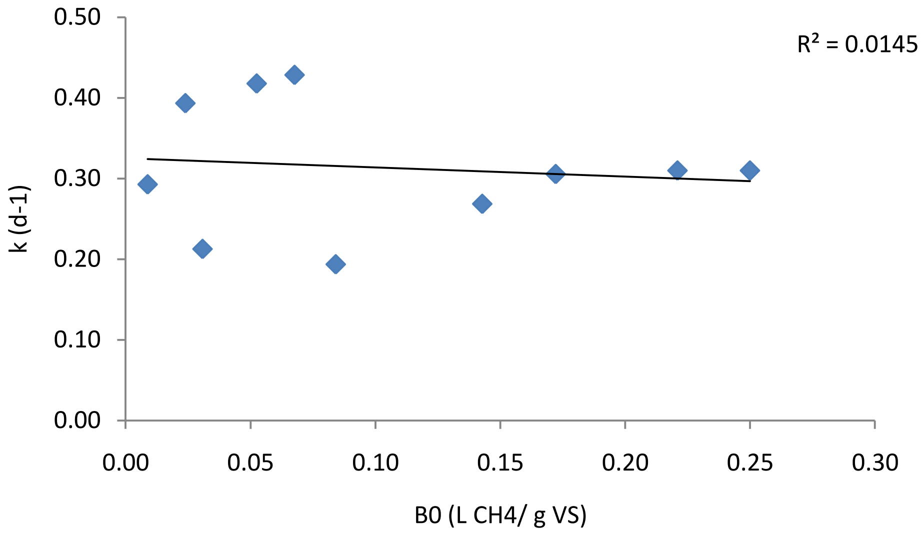


Figure 6.11 The relationship between estimated ultimate methane yield (B0) and first order rate constant (k)

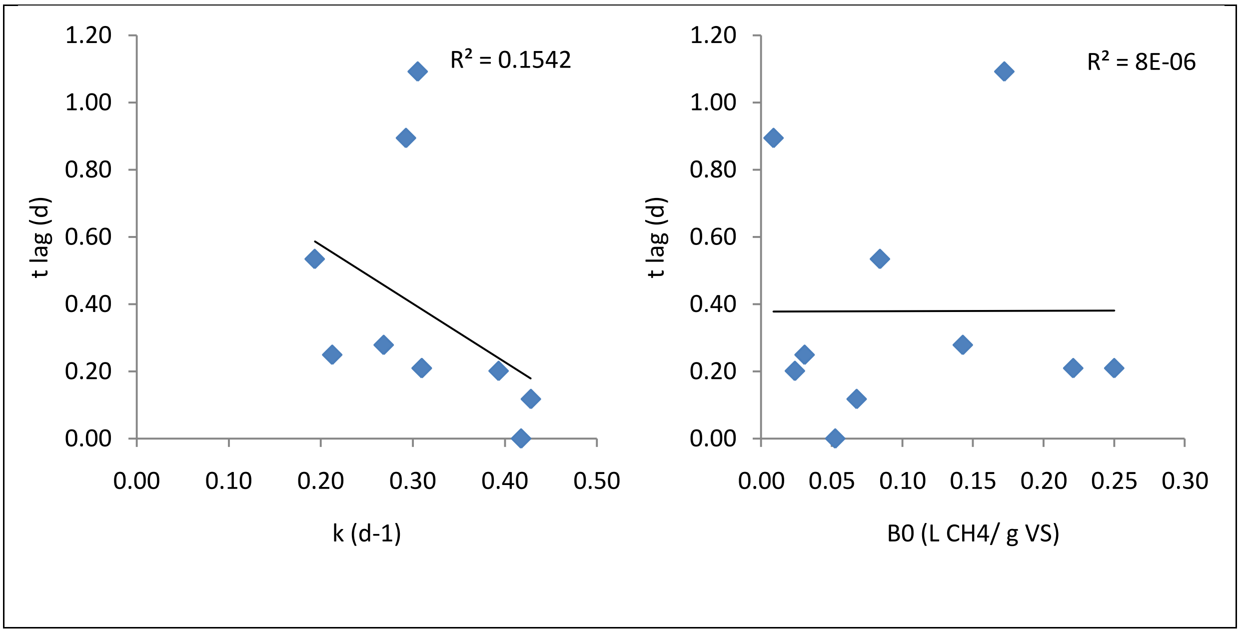


Figure 6.12 The relationship between estimated t lag to ultimate methane yield (B0) and first order rate constant (k)

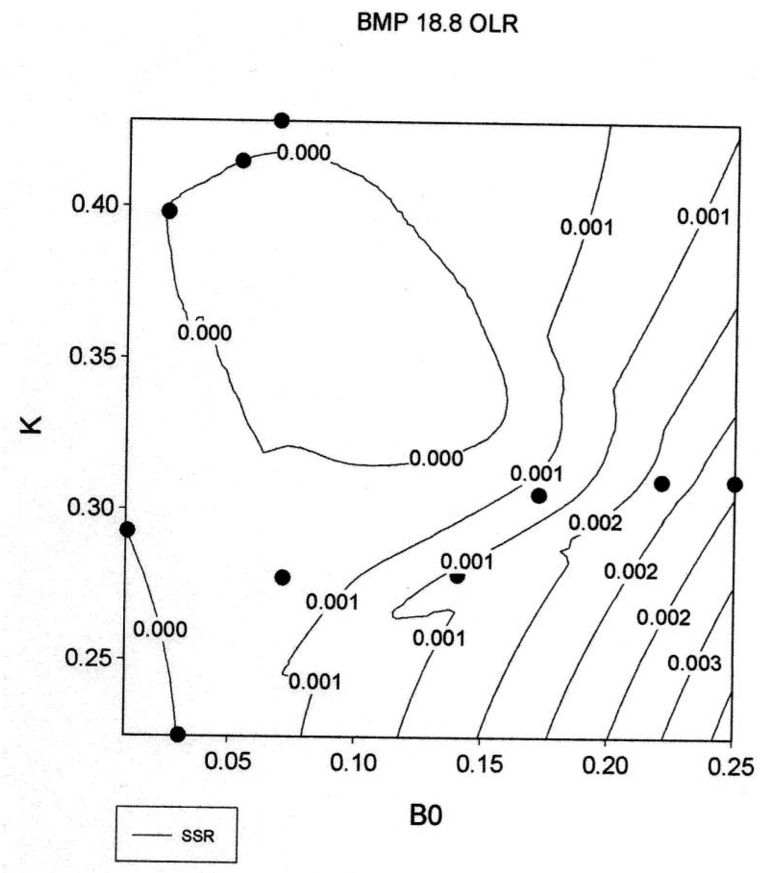


Figure 6.13 The distribution of B0, k and the sum of squared residual (SSR) for food waste at 18.8 OLR in the batch bottles. The dots are all the bottles data obtained in the experiments.

When the predicted methane yield is graphed against its experimental data, a good approximation of the fit was seen (this graph has been excluded here because the response is similar to Figures 6.6 and 6.7, hence, to avoid unnecessary repetition). This seems to suggest the suitability of the first order at forecasting reactor performance. Yet, the applicability of first order kinetics fails to highlight the possibility of conditions that could be limiting the methane production in the bottles. This is evident as the highest yield of 0.25 L CH_4/g VS is still a far cry from its theoretical value of 0.40-0.50 L CH_4/g VS.

As shown by Table 6.5, at Day 20, the tubes were still giving measurable amounts of methane, with the total methane after 20 days equivalent to about 95% of the theoretical potential. Instead, the bottles looked like the methane production had ceased after 20 days. Roughly 50% is the highest methane composition recorded in the bottle at Day 5, before production starts to lapse. The data in Table 6.5 seems to indicate that the small sized bottles which restricted to a low sample and seed volume could have been an influential factor. This issue has already brought up under Section ‘Reactor Headspace’ in Chapter 4. It appears that the bigger the sample size the shorter time it took to reach more than 50% methane composition in the headspace. In addition the methane composition in the biogas becomes higher with an increase in the sample size.

Table 6.5 Comparison of reactor and effluent conditions after 20 days incubation for tubes and bottles digesting food waste

Tube	% CH_4	Bottle	% CH_4	Bottle*	% CH_4
Test 2a	62.0	Test 5a	28.9	Test 3A	0.7
Test 2b	60.3	Test 5b	11.0	Test 3B	1.2
Test 2c	69.7	Test 5c	12.3	Test 7A	0.9
Test 3a	42.5	Test 5d	2.5	Test 7B	4.9
Test 3b	62.2	Test 5e	7.3	Test 11A	0.8
Test 3c	59.5	Test 6a	2.0	Test 11B	9.2
Test 3d	67.2	Test 6b	2.9	Control	16.2
Test 3e	64.8	Test 6c	4.0		
Test 3f	61.9	Test 6d	4.6		
Test 4a	72.7	Test 6e	1.8		
Test 4b	53.2				
Test 4c	72.0				

Note: * result in Appendix 6-c

Table 6.6 confirms that the volatile solids reduction is well balanced with the methane being produced. The pH also is at neutral conditions ranging from 7.9 to 8.2. Based on these findings, it was surmised that some other factor could be limiting the conditions within the bottles which affects the methane potential test result.

Table 6.6 The pH, experimental methane yield and volatile solids reduction of 18.8 OLR food waste digested in the bottles

Test	Measured Yield[a]	VS reduction	Total measured CH_4[b]	CH_4[c] not yet produced	Theoretical[d] CH_4 not measured	pH
	L CH_4/g VS	%	ml	ml	%	
5d	0.0085	94	23	1	98	8.2
6b	0.0238	91	64	7	95	7.9
6c	0.0299	94	80	5	93	8.0
6e	0.0534	91	142	15	88	7.8
6a	0.0680	94	181	11	85	8.2
5e	0.0820	94	221	13	82	8.0
6d	0.1392	94	371	24	69	7.9
5a	0.1705	94	460	28	62	7.9
5b	0.2134	94	576	34	53	8.0
5c	0.2488	94	672	40	45	8.0

Note: a: methane per g VS as measured in the 20 day experiments; b: total volume of methane measured in the experiments; c: Volume of methane not yet produced in 20 days (details of calculation is given in Appendix 6b); The theoretical methane volume for food waste at 0.45 L CH_4/g VS is 9108 ml, while at 0.50 L CH_4/g VS is 10120 ml. d: the theoretical methane volume – b

c) Continuous reactor

A reasonably comparable yield was obtained when the food waste was digested anaerobically in a continuous reactor under both 3.0 and 1.5 organic loading rate (OLR). This can be observed in Figure 6.14 and 6.15. For the former, an average yield of 0.313 $\pm$ 0.022 L CH_4/g VS was achieved while the latter produced an average of 0.321 $\pm$ 0.032 L CH_4/g VS. These values were obtained at steady state after a start up period of 10 days and a hydraulic retention time (HRT) of 30 days. The methane composition at this time had reached nearly 60%.

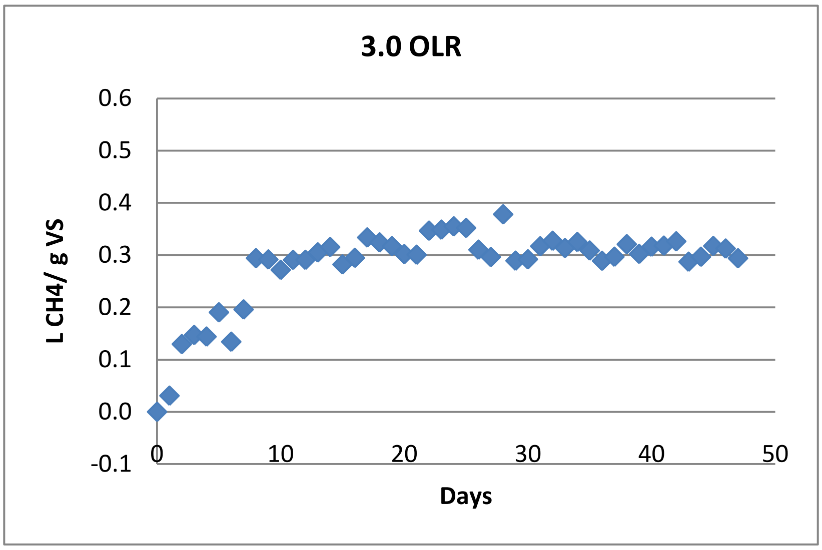


Figure 6.14 Methane yield of continuous reactor at 3 OLR food waste with 30 days HRT

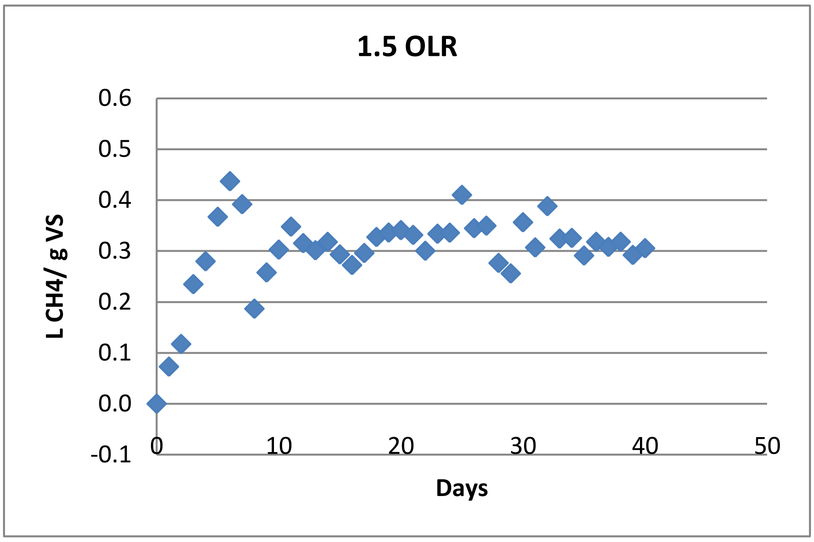


Figure 6.15 Methane yield of continuous reactor at 1.5 OLR food waste with 30 days HRT

For B of 0.313 and 0.321 L CH_4/g VS, and applying B0 of 0.40 L CH_4/g VS from the tubes, the hydrolysis rate constant for the 3 and 1.5 OLR reactors, respectively is 0.12 and 0.13 d^{-1} estimated by Equation 6.3. This coincides with the k values estimated from the batch tube tests. Table 6.7 shows the estimated k for several different values of B0.

Table 6.7 The value of k as a result of estimation using first order kinetics by varying the ultimate (B0) and specific (B) methane yield

B0 L CH_4/g VS	B L CH_4/g VS	k d^{-1}
0.17	0.153	0.31
0.25	0.230	0.310
0.35	0.272	0.115
0.35	0.312	0.277
0.35	0.321	0.366
0.35	0.334	0.681
0.40	0.313	0.121
0.40	0.357	0.277
0.45	0.290	0.061
0.45	0.352	0.119
0.45	0.401	0.275
0.50	0.334	0.067
0.50	0.466	0.275

The continuous reactor would give a rate constant of 0.28 d^{-1} following a higher specific methane production, B, of 0.36 L CH_4/g VS. The upper limit of 0.28 d^{-1} corresponds to the upper limit of k value in the batch tubes. Likewise, if the same k=0.28 d^{-1} is used together with a lower B0 of 0.35 L CH_4/g VS, a specific methane yield, B of 0.31 L CH_4/g VS can be produced. The rate constant escalates with the rise in the specific (B) and ultimate methane yield (B0) of substrate. This seems to be a result of B approaching B0 and as B approaches B0, k approaches infinity.

The area where the shading overlaps in Figure 6.24 indicates the B, k and B0 values that best describe the anaerobic process occurring in the continuous reactor digesting food waste. This occurs within k of 0.12 and 0.28 d^{-1} and B0 between 0.35 to 0.45 L CH_4/g VS. A specific methane production (B) between 0.31 to 0.36 L CH_4/g VS can generally be expected. Figure 6.16 also points out another significant behaviour; an increasing estimated rate constant corresponding to an increase in measured reactor methane yield (B), for a constant B0 and HRT.

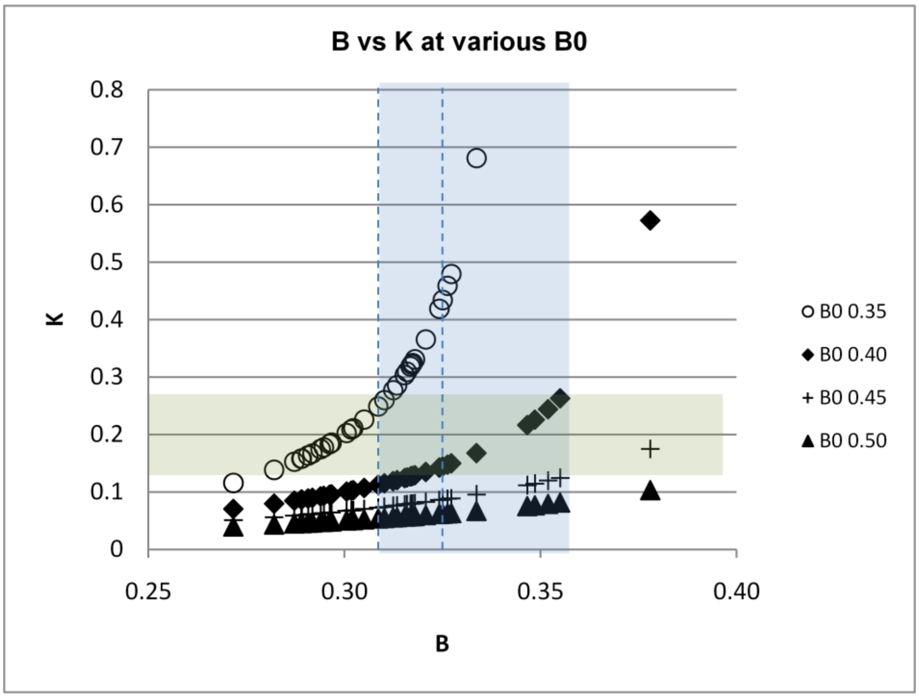


Figure 6.16 The relationship between k and B when different ultimate methane yield (B0) were applied. The vertical shaded area represents the range of B average determined from the continuous reactor at 3.0 and 1.5 OLR. The lines are the two measured CSTR B values at 0.313 and 0.321 L CH_4/g VS. The horizontal shaded area includes the range of k within the theoretical B0 average of 0.45 L CH_4/g VS as determined in the tube batch test.

6.3.1.2 Rice digestion

a) Batch tubes

The first order kinetics parameter that is estimated for rice digestion in the tubes is given in Table 6.8. The tube predicted the highest methane that could be obtained from rice is 0.39 L CH_4/g VS following a rate constant of 0.32 d^{-1}. As shown by Table 6.8, of four replicates only one measured this potential.

The first order parameter estimation using non-linear regression analysis is believed to be reliable based on the small SSR of 0.0001 to 0.0004. There is also a good agreement between the simulated methane potential to the experimental results. All rice replicates (7a-7d) in Table 6.9 achieved roughly 90% volatile solids reduction and pH neutral values with an average of 7.4. Even with a homogenous feed, the tubes showed poor reproducibility in estimating Bo, with somewhat better reproducibility in estimating k. Poor tube reproducibility at estimating the methane potential of rice could improve with an increase in the number of replicates. On the other hand, the negative correlation between B0 and k observed with food waste appeared to be less pronounced with rice.

Table 6.8 Kinetics of 11.0 OLR rice tested in tubes after 20 days

Test	B0 ($L\ CH_4/g\ VS$)	k (d^{-1})	t lag (d)	SSR
7a	0.1585	0.2408	0.2387	0.0001
7a	0.1667	0.1721	0.0000	0.0002
7c	0.2578	0.2438	0.0360	0.0004
7d	0.3880	0.3230	0.9215	0.0004

Table 6.9 The pH, experimental methane yield and volatile solids reduction of 11 OLR rice digested in the tubes

Test	Measured Yield[a]	VS reduction	Total measured CH_4[b]	CH_4[c] not yet produced	pH
	$L\ CH_4/g\ VS$	%	ml	ml	
7b	0.1580	91	1817	180	7.4
7a	0.1660	89	1903	235	7.3
7c	0.2650	88	3044	411	7.4
7d	0.3950	90	4539	499	7.5

Note: a: methane per g VS as measured in the 20 day experiments; b: total volume of methane measured in the experiments; c: Volume of methane not yet produced in 20 days (details of calculation is given in Appendix 6b)

b) Batch bottles

When rice was incubated in the bottles, the ultimate methane yield obtained a high of 0.308 L CH_4/g VS (Table 6.10). About 68% of replicates yield less than 0.1 L CH_4/g VS, while 18.5% is likely to give measurements between 0.10 to 0.20 L CH_4/g VS. Only 9.5% of the replicates have the tendency towards giving reliable representation of the ultimate methane yield (refer Figure 6.17). Figure 6.18 suggests highly uncertain rate constants when digesting rice in the bottles. No relationship can be seen between the first order parameters; the B0, t lag and k (refer Figure 6.19 and 6.20).

Table 6.10 Kinetics of 11.0 OLR rice tested in 250 ml bottles after 20 days

Test	B0 ($L\ CH_4/g\ VS$)	k (d^{-1})	t lag (d)	SSR
8d	0.013	0.279	1.177	0.000
8c	0.014	0.149	1.284	0.000
9b	0.025	1.305	0.988	0.000
8b	0.067	0.395	0.000	0.000
9e	0.074	0.628	0.000	0.000
9a	0.079	0.689	0.436	0.000
8e	0.086	0.289	0.466	0.000
9c	0.092	0.369	0.067	0.000
9d	0.111	0.502	0.227	0.000
8a	0.197	0.260	0.709	0.001
10a	0.308	0.552	0.954	0.001

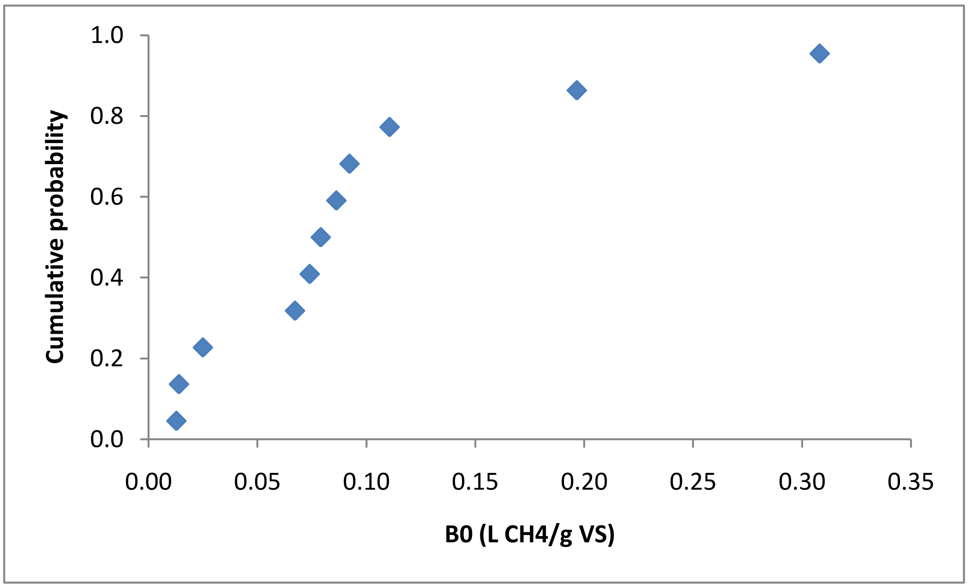


Figure 6.17 Cumulative probability graph of methane yield for 11 OLR rice measured in the 250 ml bottle after 20 days

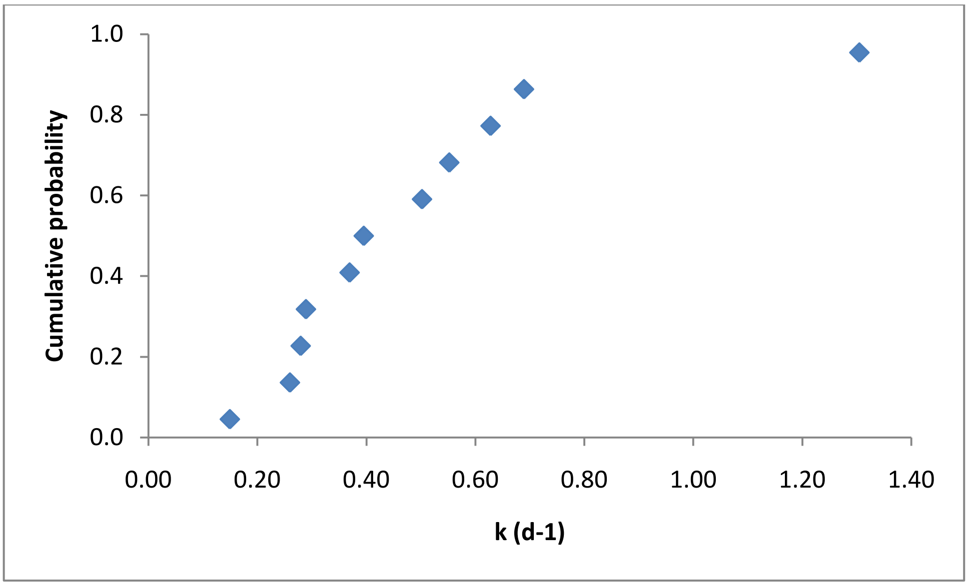


Figure 6.18 Cumulative probability graph of first order rate constant for 11 OLR rice measured in the bottle after 20 days

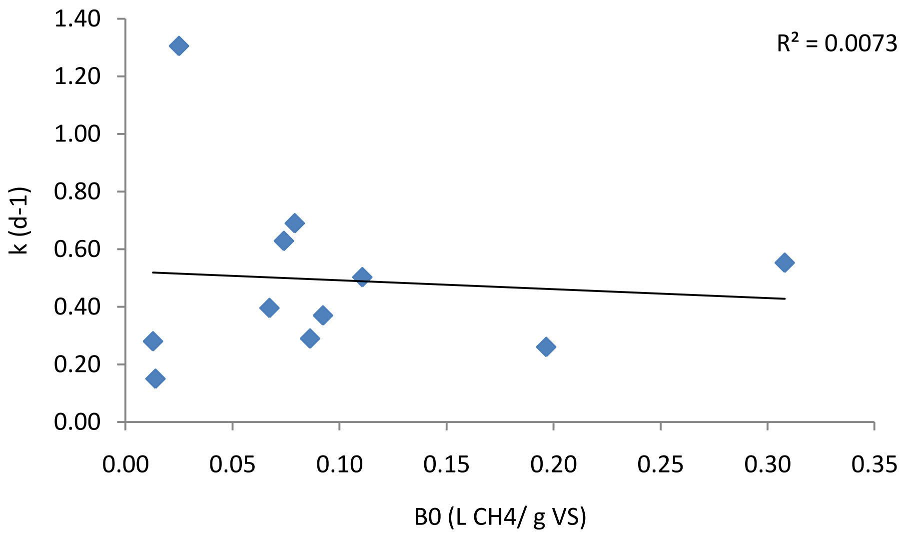


Figure 6.19 The relationship between estimated ultimate methane yield (B0) and first order rate constant (k)

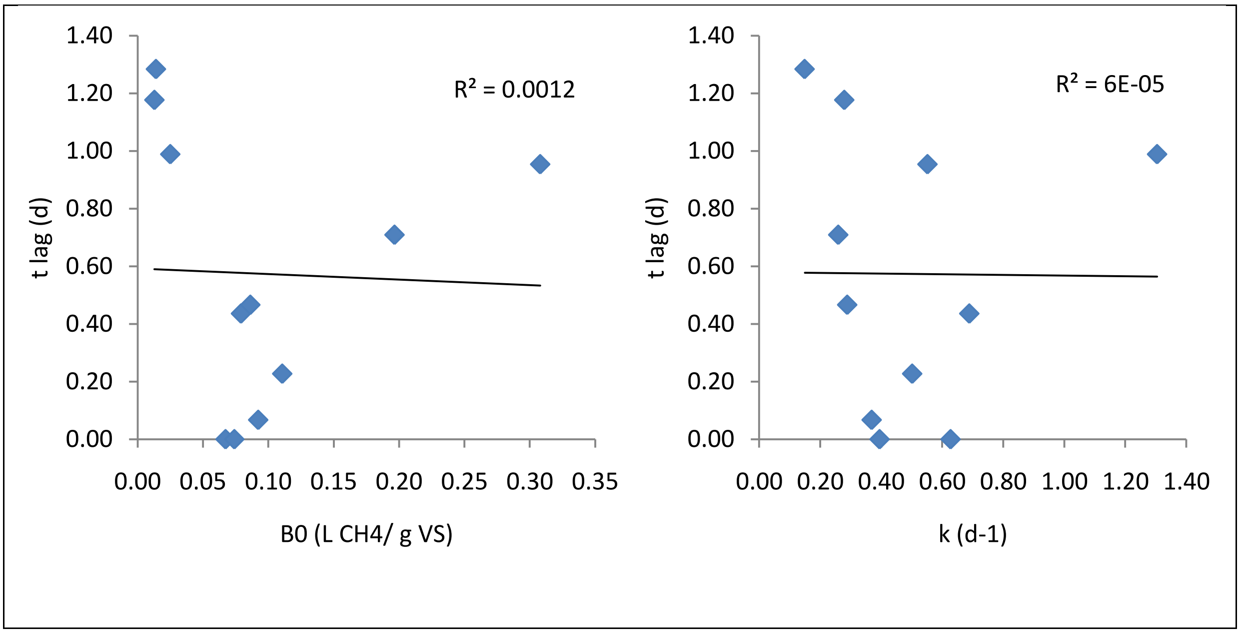


Figure 6.20 The relationship between estimated t lag to ultimate methane yield (B0) and first order rate constant (k)

The high tendency of the bottle to underestimate the biodegradation potential of rice is not expected since as illustrated by Figure 6.21, a good approximation between the experimental and simulated values are observed. The rice all reached a minimum of 93% volatile solids reduction which could reach as high as 96%. This seems to balance well with the methane production (refer Table 6.11). All the replicates retained a neutral condition (pH 6.9-8.1) throughout the incubation period. Yet, the yield of methane that can be produced per g VS is still very low and based on the food waste sample, could probably still be about 60 – 75% of its theoretical value. For some reason, the conditions within the bottle were limiting the sample degradation and methane production.

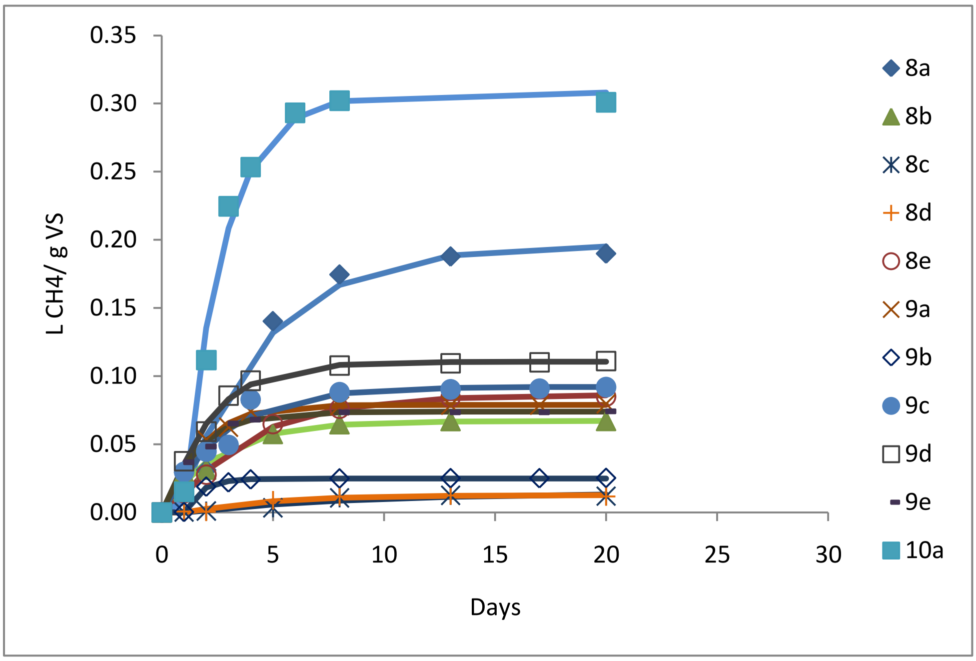


Figure 6.21 Comparison between the experimental (symbols) and its estimated methane yield over time (line) of 11 OLR rice digested in the bottles

Table 6.11 The pH, experimental methane yield and volatile solids reduction of 11 OLR rice digested in the bottles

Test	Measured Yield[a]	VS reduction	Total measured CH_4[b]	CH_4[c] not yet produced	pH
	L CH_4/g VS	%	ml	ml	
8d	0.0120	96	21	1	7.9
8c	0.0120	96	22	1	7.8
9b	0.0250	95	45	2	7.1
8b	0.0670	96	120	4	8.0
9e	0.0730	93	131	10	6.9
9a	0.0790	96	141	5	7.3
8e	0.0850	96	152	6	8.1
9c	0.0910	95	162	8	7.8
9d	0.1100	95	197	10	6.9
8a	0.1900	96	340	14	8.1
10a	0.3010	93	536	41	7.8

Note: a: methane per g VS as measured in the 20 day experiments; b: total volume of methane measured in the experiments; c: Volume of methane not yet produced in 20 days (details of calculation is given in Appendix 6b)

6.4 DISCUSSION

6.4.1 Comparison of kinetics between various systems

Food waste tested for anaerobic biodegradation in the tubes gave first order parameter values close to the ones estimated for the continuous reactor system (refer Table 6.12). The ultimate methane yield (B0) of food waste as estimated by the tubes was 0.35 – 0.56 L CH_4/g VS. This gave a corresponding rate constant of 0.12 to 0.28 d^{-1}. This B0 value (0.40 and 0.35 L CH_4/g VS) when substituted into the first order equation for a continuous system gave a similar k of 0.12 and 0.28 d^{-1}. The resulting simulation of specific methane yield, B, reactor using B0 of 0.45 and k of 0.12 and 0.28 is 0.35 and 0.40 L CH4/g VS (refer Table 6.7). These simulated B values for food waste digestion in a continuous are slightly higher from its experimental yield. The CSTR operating at 3.0 and 1.5 OLR gave a value 0.31 and 0.32 L CH_4/g VS, respectively.

Table 6.12 Food waste digestion in batch and continuous reactors

Reactor type		B	B0	k	Reliability	OLR
Tube batch	(range)	-	0.35 - 0.56	0.12 – 0.28	8/13	18.8^{1}
	(average $\pm$ stdev)		0.43 $\pm$ 0.07	0.22 $\pm$ 0.12		
Bottle batch*		-	0.25	0.31	1/12	18.8^{1}
Continuous reactor		0.32	0.40	0.12		1.5^{2}
		0.31	0.35	0.28		3.0^{2}
		0.31	0.43	0.10		

Units – B, B0: (L CH_4/g VS); k (d^{-1}); OLR ^{1}: (g VS/L); OLR ^{2}: (g VS/L. d). *For food waste digestion in bottles, the highest B0 and its corresponding k value is reported

Instead, the first order rate constant estimated by the bottles for a similar food waste sample did not return first order parameters close to the continuous reactors. The k was higher at 0.31 d^{-1}which corresponds to an underestimation of B0 to 0.25 L CH_4/g VS. If these two values are substituted into Equation 6.3, the specific methane yield that could be expected from a continuous reactor would be 0.23 L CH_4/g VS. This is a poor estimation for what could be obtained from digesting food waste in a continuous reactor.

Table 6.13 further reinforces the application of the tube at forecasting the amount of methane that can be obtained from a substrate when digesting in a continuous reactor. When rice is tested in the tubes, the B0 and k values of rice as predicted by the tubes was 0.39 L CH_4/g VS and 0.32 d^{-1}, respectively. Again, the amount of methane that could be obtained from rice when tested in the bottles is underestimated at 0.31 L CH_4/g VS (methane content 1.4%). Although no similar digestion was performed in a continuous reactor, it could be likely safe to say that the tubes stood a better probability at representing the digestion in a continuous system of a homogeneous substance like rice.

Table 6.13 Rice digestion in tube and bottle at 11 g VS/L.d OLR

Reactor condition	B0	k
Tube	0.39	0.32
Bottle	0.31	0.41

Units – B0 (L CH_4/g VS); k (d^{-1})

The same can be said for a viscous and oily liquid sample like glycerol. Data were obtained from a final year undergraduate research project (Mackay and Rowlands, Unpublished 2008) which utilised the tube to predict the minimum yield that can be obtained from glycerol. As per Table 6.14, the tubes measured an ultimate methane yield of 0.30 L CH_4/g VS at an organic loading rate of 6.5 g VS/L. d. The continuous reactor system which employed a lower OLR of 2.7 g VS/L. d, produced a higher methane yield of 0.43 L CH_4/g VS. However, because gas volume was not monitored progressively during reactor incubation, it was difficult to graph cumulative methane yield of glycerol over time, thus preventing kinetic study on these results.

Table 6.14 Glycerol digestion in batch and continuous reactors

Reactor condition	L CH_4/g VS	OLR
Tube	(B) 0.30	6.5
Continuous reactor	(B0) 0.43	2.7

Units – OLR (g VS/L. d)

6.4.2 Biodegradation rate of batch test and continuous reactor

The first order rate constant of 0.12 to 0.28 d^{-1}determined in the tubes batch tests are in agreement with the k values obtained by other researchers, though it is difficult to compare because some of the literature sources give k values for hydrolysis rather than the overall methane production rate More literature on first order kinetics on the batch biodegradation of similar degradability substrates (e.g. restaurant waste, OFMSW and FVW) would be needed before a more careful comparison of rates can be made.

Neves et al. (2008) obtained hydrolysis rate constants, assuming first order kinetics, between 0.12 d^{-1} and 0.32 d^{-1}, for restaurant waste with an excess of lipids and carbohydrates, respectively. A hydrolysis rate constant of 0.1 to 0.8 d^{-1} was recorded by Garcia-Heras (2002) when digesting proteins, lipids and carbohydrates. Cellulose digestion has been found to occur at a first order rate constant of 0.18 d^{-1} (Gunaseelan, 2009). Sewage sludge and organic fraction of municipal solid waste (OFMSW) co-digestion in a batch system reported a hydrolysis rate constant of 0.17 d^{-1} (Sosnowski et al., 2008), while fruits and vegetable had a first order rate constant of 0.12 d^{-1} and 0.13 d^{-1} (Gunaseelan, 2004), respectively. Kinetic studies by researchers like Bolzonella et al. (2005) as well as Vavilin and Angelidaki (2005) showed that the first order reaction for hydrolysis, k was 0.24 – 0.4 d^{-1} and 0.10 d^{-1}, respectively when treating municipal solid waste (MSW).

The results of this chapter show that when a reduced sample volume (the 250 ml Schott bottles) is involved, the rate constant becomes higher but the methane yield was lower. There is also the issue of instabilities seen with low methane production. As also observed by Martin et al. (1997) scale effects seem to be significant for small laboratory reactors without leachate recirculation and pH control. pH on the other hand not only acts as a general indicator for VFA production but more importantly for selective acid production during acidogenesis (Kim et al., 2008). In the work by Martin et al. (1997), the higher hydrolysis rate augmented the suppression of initial methanogenic area. This makes the system more susceptible to process inhibition if excessive VFA diffusion into the initial areas of methanogenesis occurs.

The volatile solids reduction on one instance (Test 1a, Table 6.2) only achieved 70% volatile solids reduction, much lower than the 94 % ($\pm$ 3) VS reduction average obtained by the other tests. It is suspected that in this occasion, the volatile solids measurement may not have been accurate, which would also explain the disagreement between volatile solids lost and the methane production. Utilising a small subsample (about 30 g wet weight) from the reactor for the volatile solids test may have been an ill representation of the whole reactor content.; especially since a heterogeneous sample (120 g wet weight food waste) was added at the start. This may also be due to the measurement itself which considers both the organic and inorganic matters that can burn at 550°C. A COD measurement would have been a more suitable indicator of organic matter in a substrate. However, since this research deals with a feedstock which is of a more solid nature e.g. food waste, obtaining a homogeneous, representative and analysable sample is difficult. Thus, volatile solids measurement has been used.

Neves et al. (2008) observed VS reduction in the range of 94-99% and k of 0.12-0.32 d^{-1}. In spite of that, a considerable amount of COD was observed remaining in the liquid as LCFA. This shows that there are percentages of acidified COD not converted to methane. This was also observed by Chynoweth et al. (1993) where gas production for rumen samples in biochemical methane potential (BMP) could not account for disappearance of the suspended solids. The particulate matter was thought to be converted to soluble refractory intermediates. For our case, this may have been the reason why the methane yield measured in the bottles were lower even though the VS reduction was comparable with the VS reduction of the tubes. The higher rate constant in the bottles indicated that methanogenesis may have been suppressed due to more organic acids being produced than can be converted by the methanogenic bacteria.

As shown by Figure 6.14 and 6.15, when similar substrate (food waste) was digested in a continuous reactor system, a methane yield of 0.320 L CH_4/g VS was achieved. Similarly, Angelidaki and Sanders (2004) observed that the practical yield obtained in a continuous reactor was lower than the theoretical methane potential. The theoretical methane potential generally gives a rough idea of the quality of waste and the potential methane production. According to Angelidaki and Sanders (2004) the lower yield in the continuous reactors can be due to a number of factors, namely:

a) a fraction of the substrate is utilized to synthesize bacterial mass, typically 5-10% of the organic material degraded to compensate for the lost biomass, unlike batch systems where excess biomass can be converted into methane after cell death.
b) at a finite retention time a fraction of the organic material will be lost in the effluent, typically 10%, and
c) limitation of other nutrient factors.

6.4.3 First order reliability

The SSR value of 0.0003-0.0086 in Table 6.2 for food waste digestion in the tubes when estimating the B0 and k of the first order kinetics gives a NRSME (normalised root squared mean error) of 0.07 – 0.12. The NRSME values of the tubes seem good when compared to the work of Gavala and Lyberatos (2001) who reached a NRSME of 0.05 when estimating lactose and glucose degradation using first order kinetics. The first order was able to closely fit the experimental data from the batch tests as shown in Figures 6.6, 6.7, 6.25 and 6.30. The GPR would have been highest at the start of the experiment to closely match the experimental yield, corresponding to concentration of the biodegradable substrate being at its highest.

Nevertheless, as shown in the contour plot of Figure 6.8, a few of the estimated B0 and k were outside of the ‘acceptable’ range. This ‘acceptable’ range is defined by B0 of 0.35-0.56 L CH_4/g VS and k of 0.12 to 0.28 d^{-1}. Good contact between biomass and substrate has been shown to be requirements for hydrolysis (Vavilin et al., 2004). Factors such as the physical state and structure of the substrate and its accessibility for hydrolytic enzymes influence the workability between biomass and substrate. Song et al. (2004) found the rate of microbial attachment to the substrate depends on the type of micro-organisms. This is in parallel to the findings of Neves et al. (2008) and Jensen et al. (2007) who attributed the variability in the kinetic constants to the choice of inoculums. Clearly, the quality of the inoculums plays an influencing part in a batch anaerobic digestion test as it can affect the biodegradation kinetics.

Figure 6.4, 6.12 and 6.20 demonstrates that no relationship can be drawn between the experimental lag phase to the B0 or k value. However, it has been shown in Section 6.3.1.1 that discounting the lag phase fit when estimating for B0 and k can exhibit a noted effect on the estimated rate constant. The k values tend to be overestimated by up to 14%. The B0 estimated in less affected (tendency for 3% overestimation) if t lag is not considered in the model fitting. Even so, the fact that different lag phase values led to varied hydrolysis rates and yields, supports the notion that micro-organisms adapt differently. This may further reinforce the finding of this research regarding the influence of microbial adaptation to the substrate degradation. The following were thought to be factors for lag phase (Gavala and Lyberatos, 2001):

a) the need for sufficient growth of the type of bacteria responsible for the consumption of the particular substrate,
b) the need for build-up of the necessary enzyme(s) for substrate consumption or
c) a combination of (a) and (b).

First order states that the velocity of the process is controlled by the substrate concentration (Mata Alvarez et al., 1990). The first order kinetics for the continuous system especially in Figure 6.16, show a linear relationship between the first-order constant and methane yield of the continuous reactor where a higher k leads to an increased B, at constant residence time. When a higher B0 value from the batch test was applied, this behaviour repeats, increasing parallel to B0 value. On the other hand, the kinetics in the bottle confirmed that hydrolysis may proceed at a rate too fast. This signifies excessive VFA may be produced and methanogenesis, the stage where methane is converted from VFA, can be hampered. This is especially a concern when an easily degradable substrate is involved. Food waste consists of soluble organics which rapidly convert to VFA at early stages of hydrolysis (Heo et al., 2004). This behaviour was also observed by Neves et al. (2006). When hydrolysis was faster, the methane yield was lower. Neves et al. (2006) suggests that intermediates formed during the hydrolysis step were likely toxic to the methanogenic population. This phenomena might help explain the restricted bottle results with this research.

6.4.4 Reproducibility of test results

The applicability of the bottle at determining the ultimate methane potential of solid organic waste was tested for two substrates, food waste and rice with the latter being more homogenous. The initial run where conditions in the bottles were identical to the tubes (no supplementation, primarily substrate and seed) saw a restricted methane yield. Thus, supplements such as nutrients as well as buffer solution were added and the same test repeated. However as the results in the Appendix 6-c show, no benefit was observed and methanogenesis in the bottles was hampered for some other reasons. Although no oxygen peaks was observed in the chromatogram, there is the potential of oxygen being present at levels sufficient enough to harm the methanogens. The solutions could be at high enough oxidation potential to depict a negligible oxygen on the GC or that O_2 could have been consumed prior to analysis. Further efforts in reducing oxygen ingress are suggested when conducting tests in bottles. Methods such as adding a small amount of reductant (sulphide) once in a while and/or applying a blob of silicone rubber sealing to enhance the re-sealing ability of the rubber septum, could be tried to see their effect on methane generation.

The 1.5 L working volume with the tube catered for a larger sample size compared to the bottle with merely 200 ml working volume. For the same OLR of 18.8 g VS/L. d., a 120 g (w/w) food waste sample in the tubes yielded 50% more methane per g VS compared to 16 g (w/w) of a similar sample in the bottles. The former produced up to 0.499 L CH_4/g VS as tested in the tubes while the latter produced a maximum of 0.249 L CH_4/g VS in the bottles. The use of a larger sample size seems to generate a higher methane yield. It was also the same case with rice at 11 g VS/L. d OLR. The tube allowed 50 g (w/w) sample which gave a highest methane yield of 0.397 L CH_4/g VS. The bottle containing just 7 g (w/w) rice produced a maximum of 0.193 L CH_4/g VS. Again the larger sample size in the tube increased the amount of measured methane yield by 50% for the same OLR. The use of a large sample in the tube, almost 7 times of that for the Schott bottle, increases the test precision by 50%. The small sample volume of less than 20 g (w/w) in the Schott bottles underestimates the measurable methane yield for both food waste and rice by half. However, as mentioned above, possibility of oxygen contamination limiting methane generation could also be a factor.

The sample pre-treatments as investigated in Appendix 6-c where food waste sample was dried and ground did not increase the methane yield of samples measured in the bottles, although it appeared to improve the reproducibility between duplicates. The effect of sample pre-treatment on result reproducibility should be confirmed further to outweigh the extra effort required and warrant its necessity when determining the methane yield of a sample in a batch test. Hansen et al. (2004) seemed to promote extensive sample pre-treatment when analysing anaerobic degradation of food waste in their batch set-up. In their work, samples were first blended in a large industrial blender without water to reduce the particle size and mix the sample. Then, water was added and sample further blended. This was followed by transfer to a high speed blender and again blending for 5 minutes. Nonetheless, other researchers like Chynoweth et al. (1993), Shanmugan and Horan (2009), and Charles et al. (2009) were also able to achieve similar methane yield from a comparable substrate without undergoing any sample pre-treatment apart from a simple homogenization.

Food wastes which were dried and shredded to 2-6 mm size produced methane that was at par with another reactor fed with similar non shredded feedstocks (Forster-Carneiro et al., 2008).

An improvement to biogas production rates was also absent when analysing the methane potential of shredded paper and cardboard (Pommier et al., 2010). It seems the structure of the materials favours the biodegradation process (Forster-Carneiro et al., 2008). Although shredding strongly affects the macrostructure of the waste, it did not however improve the enzyme accessibility to cellulose nor does it favour the surface bacterial colonization (Pommier et al., 2010). Therefore, shredding of materials for digestion is thought to be unnecessary especially where high inoculums levels are provided. The reasoning behind sample drying and size reduction prior to digestion could probably be more related to hygiene purposes more than others particularly if the digestate is to be spread on land. As required by the European Union Animal By-Products Regulation, particle size reduction of less than 12 mm and sanitization by heating at 70°C for 1 hour is successful in reducing pathogens such as Salmonella to undetectable levels (EC, 2002).

Stabnikova et al (2008) show that food waste which was first frozen overnight at -20°C then thawed for use in an anaerobic digestion exhibit a similar effect to samples which were thermal pre-treated at 150°C for 1 hour. It seems the structure of food waste after freezing/thawing became looser, suggesting the occurrence of cell disruption followed by an increase of dissolved organics concentration. Consequently, a faster methane production was observed with the possibility of cutting batch incubation time by 42%.

With the tube, the results are obtained by examining the substrate without any pre-treatment like drying or fine shredding of sample. Just like the research of Stabnikova et al. (2008), the food waste in this research was thawed overnight after being frozen at -20°C for some time. The only action taken to the sample prior to analysis in the tube is blending to small pieces to obtain a homogenous sample. The freezing/thawing of food waste in this research may have eliminated the requirement for other pre-treatment since cell disruption has already occurred, enabling sample decomposition and subsequent methane production. In addition, no other supplements like nutrient or trace elements were required. Another advantage of the tube is in terms of workload and time spent. For determining the gas production in the Schott bottles, the Gas Chromatograph (GC) is required, which involves procedures like:

(i) method development and calibration

(ii) presence of sufficient gas sample in the bottle. This may be difficult for substrates with low gas production where gas has to accumulate first, which may mean sampling may only be done after a certain period e.g. 7 days.

(iii) roughly 5-10 minutes is required per sample to undergo GC analysis and obtain a chromatograph of the biogas composition. This may not seem excessive for one sample. However, when analysing 18 bottles like in this research, over 2 hours would be needed to analyze all.

The gas meter that was used with the tube enabled a much shorter analysis time of 20 minutes for all 18 tubes. Only a minute is required to determine the gas contents from one tube. As the gas analysed is recirculated from the tube headspace, no gas accumulation is needed. This means gas measurements can be taken every day (if required) to obtain a more representative graph.

It has been shown in Chapter 4 that substrate does not control reproducibility since rice which is heterogeneous in structure gave a varied methane yield for a similar sample loading and test condition. Neither was seed as not only was methane yield of waste sample varied despite the seed use consistency, even the seed blanks performed variably. It was learnt in Chapter 4 that the OLR at which testing was conducted has an effect on replicate variability, with reproducibility improved at an OLR further away from its maximum tolerance.

It is found that irreproducibility of replicates does not largely affect the potential of obtaining reliable kinetic estimates from the tubes. As shown by Figure 6.1, despite the replicate variability, less than 35% of replicates is likely to estimate B0 values that is not representative of the sample, in this case food waste, This is also the situation with the first order constant estimation. Even though irreproducibility was observed with the replicates, the likelihood that a false estimation for k to be given, is less than 37%. It does not seem that either estimates of B0 or k are more in need or replicates than the other. Both will benefit equally from more sample testing.

6.5 CONCLUSIONS

The first order rate constant (k) value obtained in the tube is similar to the k value of the continuous system when the ultimate methane yield of close to 0.45 L CH_4/g VS is used. This shows that the tube tests were able to portray the food waste biodegradation occurring in the continuous system. The continuous procedures closely simulate full-scale anaerobic operation; however, they are costly in terms of facilities, equipment, time and personnel. The batch bioassay techniques are not restricted by these limitations, and they allow the evaluation of a wide range of variables.

This research recommends using kinetics rather than experimental data alone (e.g HRT, OLR and methane yield) when planning and designing an industrial plant. The benefits of kinetics includes (i) predicting the methane potential when operating conditions are changed for example varying HRT or substrate concentration (ii) forecasting any inhibition possibilities by examining the kinetics parameters and (iii) deciding a suitable reactor size to avoid overdesign and unnecessary building, maintenance and operating costs.

Inoculum quality, substrate concentration and good contact between them may affect and determine the suitability of applying first order kinetics. The first order seems suitable when the anaerobic process is healthy. Here, the methane yield of the substrate is governed by the hydrolysis rate because methane production is uninhibited. However, if digestion proceeds when substrate or inoculum cause an inhibition of methane production, then the hydrolysis rates tend to be too high for healthy anaerobic digestion. The high k value implies a risk that VFA was being produced faster than can be converted into methane. This may be a function of the substrate volume or size and inoculums quality.

6.6 SUGGESTIONS

In view of the findings of this chapter several aspects are suggested when conducting a similar study. These are:

1. To obtain enough experimental data for calculation of the rate constant it is essential that the batch reactor is sampled at smaller intervals in the beginning of the experiment than at the end. Nevertheless, enough data has to be gathered after long time periods to establish the biodegradability of the substrate.

2. Whenever permissible, to conduct effluent sampling regularly during the course of the incubation period. This is so that the kinetics modelled based on gas production can be further validated. First order kinetics can be modelled based on the progression of substrate degradation or organic acids production and uptake. One way to do this is by taking small amounts from the batch reactor effluents provided that this is done under minimal exposure to oxygen and a large reactor effluent is available in the first place. Alternatively, a series of similar batch reactors can be set up and its contents analysed and not reused. The reactor effluents can be tracked for VS reduction, VFA production and uptake and/or SCOD progression.

CHAPTER 7

SUMMARY AND CONCLUDING STATEMENTS

7.1 OVERVIEW

This thesis has examined the current methods of assessing the anaerobic decomposition and methane potential of solid organic waste. Although many laboratory-built and commercial tools are available, several weaknesses were apparent which warrant a further study. Traditionally these batch tests are used on liquid samples, which works out fine considering the material is devoid of large particulates and are largely homogeneous. With the current direction of anaerobic digestion as a sustainable waste management option and energy generator, more and more solid organic samples are being used as feedstock. The same batch tests are being utilized to analyse these solid samples. Because of many bottles' small capacity and narrow neck, samples are extensively homogenised and only a small sample volume can be accommodated. The effect of such sample disruption with a restricted sample volume was clarified in this thesis. Another concern has been the lack of kinetics applicability of kinetic values obtained from batch tests to help simulate the performance of a similar substrate digested in a continuous reactor. As widely known, the majority of full-scale anaerobic reactors are operated in continuous mode.

As a consequence, an anaerobic batch respirometer tool or 'tube' to was developed here to improve the current state of analysing organic samples. Of more significance, the tube has been shown capable of estimating the methane potential and degradation rate of a substrate if treated in a continuous reactor. This Chapter presents the conclusions drawn from achieving such research objectives.

7.2 IMPROVED BATCH TESTING

The feature of the tube design was the large vessel opening (10 cm diameter) and reactor space (3.4 L). These elements enable samples to be analysed in big volumes and without tedious sample pre-treatment. Food waste, the main substrate in this research, can be digested to a maximum of 240 g (wet weight) in the tubes. To estimate the amount of wet sample that should be loaded, the volatile solids content of the sample needs to be known. Say for example a sample contains 60% VS, based on Figure 7.1(a), the amount of sample to be loaded is roughly 52 g. Initially, it is best to vary this amount, using values higher and lower than recommended. This is to be certain that substrate is provided at the optimum and would not be limiting. Therefore, a researcher might want to try 26, 52 and 78 g wet weight sample loading. Based on a fixed reactor volume of 1.5 L, the corresponding organic loading rate should be 8.77, 13.18 and 17.59 g VS/L. d (refer Figure 7.1 (b)).

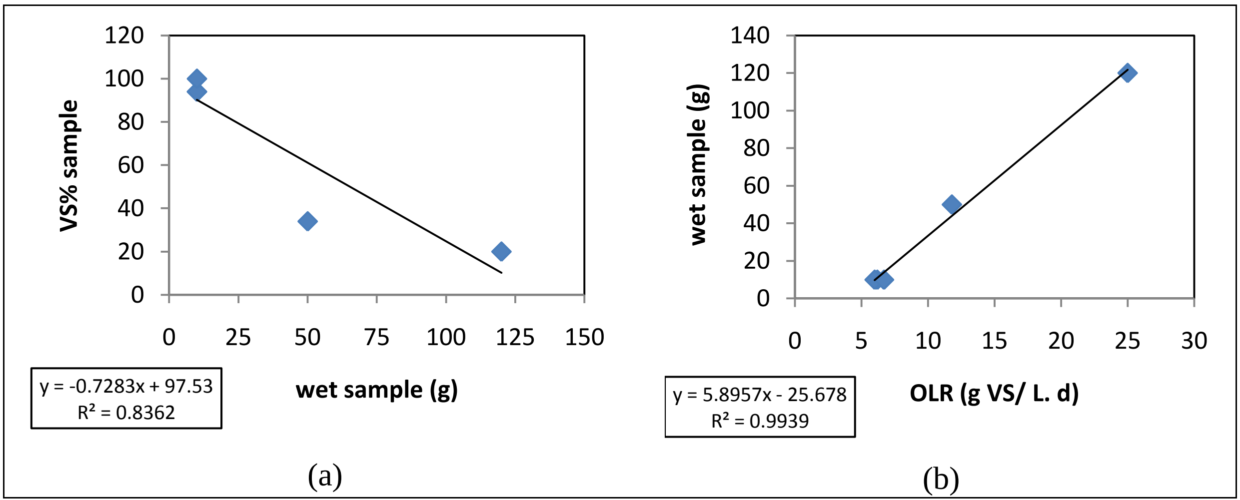


Figure 7.1 The estimation of sample loading (a) based on VS% of sample and the (b) corresponding OLR values. Data compiled from food waste, rice, starch, glucose and cellulose digestion in a 1.5 L DSS seed. The OLR was the highest obtainable before reactor souring and not based on highest yield due to the large variability.

A large sample size needs to be followed by a sufficient volume of seed (in this case 1:12.5 v/v ratio) to avoid process souring due to overloading and the accumulation of volatile fatty acids (VFAs). Based on the VS content of the sample, the volume of seed in terms of inoculum to substrate ration ($R_{I/S}$) can be roughly estimated using Figure 7.2. For instance, for the 60% VS sample, an inoculum to substrate ratio of 0.81:1 may be appropriate.

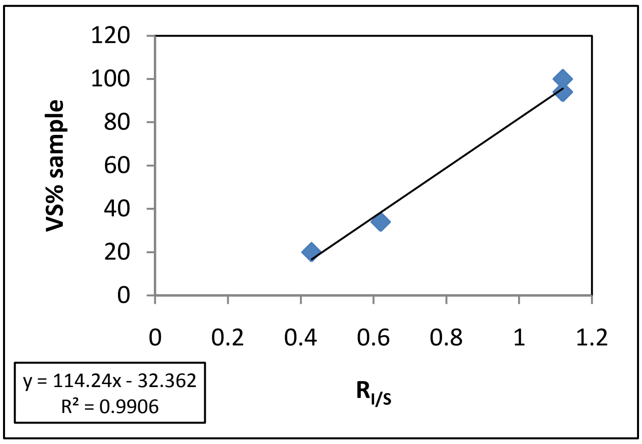


Figure 7.2 The determination of the ratio of inoculum to substrate based on the VS% of sample. This Figure was built from the digestion of rice, food waste, starch, cellulose and glucose in a 1.5L DSS seed.

Predigesting the seed first for 5-7 days can help reduce the background gas production which could otherwise blur the methane contribution from the substrate. Seed pre-digestion involves storing seed (in this case collected from a wastewater treatment plant) in a temperature controlled room at 35°C without mixing or feeding for 5-7 days.

The use of a large sample volume, up to 15 times more in the tube than in the 250 ml Schott bottle benefitted the methane potential determination. For the same 18.8 g VS/L. d organic loading rate (OLR), the tubes generate a higher methane yield compared to tests in bottles and saw an increased test precision by 50%. The small sample allowable in the bottles underestimated the methane yield of substrates by half, which was thought to be influenced by inhibition resulting from an extreme degradation rate. Extensive sample homogenisation like drying at 100°C overnight and grinding to smaller particle sizes can help to control replicate variability; however, it did nothing to enhance the methane yield of substrates. The freezing and thawing of the food waste prior to use may have caused sample solubilisation to the same effect of thermal pre-treatment.

This research found the methane yield of a food waste can be achieved close to its theoretical value with minimal workload, no supplementation as well as a short incubation time when using the tube reactors. Mixing, manually, need only be done once a day or even once a week, enough to encourage the contact between substrate and bacteria, without detrimental effect to the substrate degradation. In fact, food waste methane yield was down by 20% when tubes are mixed hourly. The enhanced distribution of VFA into methanogenic centres caused by frequent mixing may have hampered the methane production. Trace elements and buffers were not necessary during incubation and if provided in excess, can lead to a reduced methane yield. It is believed that successful digestion of substrates in the tubes was achieved following a suitable OLR, and the seed and substrate having sufficient nutrients for microorganism growth. The methane potential results can be obtained as quickly as four days to a longer duration of 20 days, depending on the finding of interest. For a simple test like establishing a workable OLR and using a mixed food waste as substrate, a 4 days test will give a 50% degradation while a 6 days represents 75% of the maximum yield. A 20 day test is usually more suitable for kinetics study because this is when most of the sample has been degraded (95%) and gas production has levelled out.

This research has addressed the issue of test reproducibility and found that type of substrate does not control the replicate variability. The loading at which the substrate is tested as well as seed quality seem to have an influence over replicate variability. Irreproducibility increases the further away the substrate is tested from its maximum OLR tolerance. Seed could be affecting test reproducibility by the distribution of inocula in reactors not being sufficiently homogeneous, or seed being inhibited, or of other reasons produced too little methane.

7.3 IMPROVED BENCH-SCALE CSTRS

A guideline for starting, operating and maintaining a single-stage mesophilic continuous reactor was established. The guideline is simple yet adequately informative with the intention of helping first-time operators to successfully initiate and monitor a continuous reactor.

It is recommended that for a simple single stage continuous reactor operation, a hydraulic retention time (HRT) around 30 days is chosen. A shorter HRT could lead to microbial or micronutrient washout. The amount of feedstock to be loaded per reactor volume or organic loading rate (OLR) can be established by (i) researching the literature for example of a similar substrate, and/or (ii) conducting batch tests on similar samples. To achieve the set HRT and maintaining the established OLR, the feedstock is to be diluted or by adjusting the feed/waste frequency.

A reactor should be started primarily on seed followed by regular substrate feeding. During the first few days of incubation, volatile fatty acids will be produced and accumulate due to the breakdown of substrate to simpler forms. However, more often, the produced VFAs are not being converted to methane fast enough due to the slow doubling time of methanogens. It is therefore important that steps to calm this acid burst be implemented during this critical time especially if the reactor content are being exchanged frequently. This research has seen the benefit of adding phosphate buffer in each feeding from day 1 until the 9^{th} day of reactor operation. A suitable reactor OLR will eventually self regulate and enter steady state within 15 days producing about 50% methane content. Regardless of the buffering, an unsuitable OLR will see the reactor failing with pH less than 6.5 within a week.

Once the reactor is operating, signs of a stressed or inhibited reactor should be looked for. A troubled reactor will generally show signs of an increasing VFA concentration, followed by drop in pH, methane percentage and alkalinity. Regular monitoring of pH and methane content is highly recommended as not only are the measurements simple and fast to take, their response is also reliable and rapid. Methane content can drop by 10% while pH by 0.15-0.25 in a day, following a troubled condition. For mixed food waste, a healthy reactor would normally operate at methane about 60% and pH around neutral (7.0). Microbial and/or micronutrient washout, sudden temperature drop and excessive ammonia concentrations can be a cause of reactor deterioration.

Simple actions such as (i) temporarily stopping feeding for a day or one week, (ii) addition of extra bacteria and/or (iii) methods to encourage bacterial growth can help to revive a failing reactor. These actions build again upon the concept of calming the acid burst within the reactor and at the same time allow the methanogenic population to recover. This is because when a reactor is stressed, this is usually linked to an imbalance between process of hydrolysis/acidogenesis and methanogenesis. Therefore it is recommended that trace element be added on a casual basis after a long reactor operation e.g. 80 days. In addition, a secondary reactor should be run concurrently as a back-up reactor or to serve to supply external bacteria to another reactor. Recovery is usually faster for a low OLR reactor compared to a reactor operating at high OLR.

7.4 COMPARING BATCH AND CONTINUOUS SYSTEM KINETIC PARAMETERS

As a point of rule, the theoretical methane potential of the substrate should be known. This is so that the researcher would be confident the values obtained through the batch and continuous experiments are 'correct'. The first order kinetics, due to its simplicity, was used to model the degradation of food waste under both batch and continuous system. The result of estimation indicated the comparability between kinetic parameters estimated by the tube and continuous reactor in terms of a similar first order rate constant (k). Both reactors estimated a k value between 0.12 – 0.28 d-1, with the continuous reactor also utilising the ultimate methane yield (B0) value of 0.35 and 0.45 L CH4/g VS determined from the batch tube tests.

It seems by examining a sample in the tubes and estimating its k and B0 value, the methane yield and degradation rate of a similar sample in a continuous reactor is likely to be reasonably forecasted. The tube estimated food waste can produce 0.35-0.40 L CH_4/g VS in a continuous reactor while the experiments verified about 0.32 L CH_4/g VS is producible. The kinetics agreement between the tubes and continuous reactors are of significant finding since this means a reactor designer can save time and money without having to run a lab-scale continuous reactor which requires constant monitoring, content exchange and long operation.

By establishing the retention time from substituting B, B0 and k into Equation 7.1, the reactor volume can be calculated by iterating Equation 7.2. Take for example 500 m^3 of substrate need to be treated daily. A retention time of 30 days will require a reactor volume of 15 000 m^3 (500m^3/d * 30 d). If the substrate contains 20% VS, the OLR should be 6.7 kg VS/m^3 d calculated using Equation 7.3.

Eqn 7.1 (source: Mata-Alvarez, 2003):

$$B = B0\left(\frac{k.HRT}{1 + k.HRT}\right)$$

Eqn 7.2 (source: Mata-Alvarez, 2003):

$$HRT = \frac{V}{Q}$$

where HRT: the ratio of the reactor volume to the flow rate of the influent substrate (day), V: reactor volume (m^3) and Q: flow rate (m^3/day).

Eqn 7.3 (source: Mata-Alvarez, 2003):

$$OLR = \frac{Q * S}{V}$$

where OLR: is the substrate quantity introduced into the reactor volume in a given time (kg VS/m^3 reactor day), Q: substrate flow rate (m^3/day), S: substrate concentration in the inflow (kg VS/m^3) and V: reactor volume (m^3).

It is advised that first order kinetics be used when anaerobic process is healthy but avoided when process is not. This is because, under healthy conditions, the methane yield is governed by the hydrolysis rate because methane production is uninhibited. This means the model illustrates the behaviour of increasing gas production over time during the initial stage proportional to the amount of organic matter present. However, if inhibition occurs, the hydrolysis rates tend to be too high, indicating VFA was being produced faster than can be converted into methane. It seems the inocula quality, substrate concentration and good contact between them may affect and determine the suitability of applying first order kinetics.

7.5 FUTURE WORK

To aid in improving the quality of this research, an understanding of the limitation observed throughout the study are presented. These limitations are areas recommended for further investigation in the future.

1. There were difficulties associated with obtaining a representative methane yield when substrates (food waste and rice) were digested in the 250 ml Schott bottles with rubber septum. At this point it is unsure what could be restricting the methane production of the samples, whether technical (reactor configuration), biochemical or microbiological factors are at play. It is certain however that, oxygen ingress as well

as nutrient deficiency, loading rate or feedstock structure was not influencing the reactor performance.

The testing of solid substrate in a bottle reactor and rubber septum lid should be repeated with modifications done to the set-up. If methane production is still hampered, factors other than the ones identified above should be investigated and addressed. It would be interesting to learn more on this issue and offer advice to other potential users of batch bottle reactors.

2. The findings on factors affecting test reproducibility, especially concerning sample condition and seed distribution and inhibition, provide an insight to factors that control replicate variability; however, further work is suggested. In addition, future application of a batch methane potential test could also benefit from the use of methods that are proven to increase the margin of test reproducibility.

3. This research is also limited by use of substrate, particularly food waste and meat, that has been frozen and thawed which could have led to the varied response in methane generation and substrate decomposition. Researchers referring to this work should be aware of this and note that a different behaviour might result from the use of fresh or unfrozen substrates. This also serves as the ground for further study, for example how far the value of methane potential given by a frozen/thawed feedstock differs from a fresh one. The result is of interest especially for tropical countries where storage of waste in coolers is unavoidable to reduce odours and vermin attacks.

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Determination of the Theoretical Methane Potential (TMP) of Food Waste

The chemical composition of the food waste sample used throughout this research is as Table A1.

Table A1 Chemical composition of the food waste sample
(results based on *as received* basis)

Percentage %						
DM	Ash	Fat	Protein	NDF	ADF	Lignin
23.5	4.5	2.4	3.4	4.9	3.3	1.6

DM: Dry matter. Acid detergent fibre (ADF) is a measure of cellulose and lignin. Neutral detergent fibre (NDF) is a measure of total insoluble fibre and includes cellulose, lignin and hemicellulose. It is a better indicator of total fibre than ADF.

The theoretical methane potential of food waste can be calculated using the Buswell's formula (Angelidaki and Sanders, 2004):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \longrightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2$$

In order for the Buswell's formula to function, the chemical and/or elemental compositions of the substrate need to be known. Knowledge of the substrate's chemical and elemental fractions allows the determination of theoretical methane potential via three ways: (i) from protein, fat and carbohydrate distributions (ii) use of the maximal theoretical methane yield constant (350 ml CH_4 (STP)/g COD), and (iii) substrate's elemental composition. Each of these estimations is detailed below.

(i) Using the known chemical fractions

From Table A1, food waste contained 3.4% protein, 2.4% fat and 13.2% carbohydrate (carbohydrate is found indirectly from 100%-protein-fat-moisture-ash = 100-3.4-2.4-76.5-4.5). Chemical formula of protein is $C_5H_7NO_2$, fat $C_{57}H_{104}O_6$ and carbohydrate $C_6H_{10}O_5$. Using Buswell's formula, the theoretical methane potential of each chemical fraction are calculated and later summed to equate the TMP of the substrate.

Protein ($C_5H_7NO_2$):

L CH_4 (STP)/g VS = (2/2 + 7/8 – 2/4)22.4 / 12(5) + 7 + 16(2) = 0.311

Fat ($C_{57}H_{104}O_6$):

L CH_4 (STP)/g VS = (57/2 + 104/8 – 6/4)22.4 / 12(57) + 104 + 16(6) = 1.014

Carbohydrate ($C_6H_{10}O_5$):

L CH_4 (STP)/g VS = (6/2 + 10/8 – 5/4)22.4 / 12(6) + 10 + 16(5) = 0.415

L CH_4 (STP)/g sample = 3.4 % protein + 2.4 % fat + 13.2 % carbohydrate

= 0.034(0.311) +0.024(1.014) + 0.132(0.415) = 0.09

A gram of food waste sample contains 0.19 g volatile solids which are made of 0.034 g protein, 0.024 g fat and 0.132 g carbohydrate.

Therefore,

TMP of food waste $= \frac{0.09 \frac{L\ CH4}{g\ VS\ sample}}{g\ sample}\ x\ \frac{1\ g\ sample}{0.19\ g\ VS}$

= **0.45 L CH4 (STP)/g VS**

The TMP determination via method (ii) and (iii) requires the elemental composition of the food waste. As this is was not analytically determined, the elemental fractions of carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) has to assumed based on known protein, fat and carbohydrate fractions. The C,H,O and N of food waste is calculated as the following:

- 3.4 g protein has 5 carbon, 7 hydrogen, 1 nitrogen and 2 oxygen which is equivalent to 33.3% C, 46.7% H, 6.7% N and 13.3% O by composition.
- 2.4 g fat has 57 carbon, 104 hydrogen, 6 oxygen which is equivalent to 34.1% C, 62.3 % H and 3.4 % O by composition.
- 13.2 g carbohydrate has 6 carbon, 10 hydrogen and 5 oxygen which is equivalent to 28.6 % carbon, 47.6 % hydrogen and 23.8 % oxygen.

Therefore, by equating the percentages of the C, H, and O of each component to their weight, protein has 1.13 g C, 1.59 g H and 0.45 g O. Fat has 0.82 g C, 1.50 g H and 0.08 g O, while carbohydrate has 3.78 g C, 6.28 g H and 3.14 g O.

Summarising all the respective elements then gives the following formula for the food waste: $C_{5.73}H_{9.37}O_{3.67}$. This is the elemental formula used when determining the theoretical values for (ii) and (iii).

(ii) Maximal theoretical methane yield constant (350 ml CH4 (STP) g COD) (Buffiere et al., 2006)

To make use of the above constant, the COD of the substrate is needed. The COD value of food waste can be estimated based on the substrate’s volatile solids composition.

$$C_{5.73}H_{9.37}O_{3.67} \quad + \quad 6.23\ O_2 \longrightarrow 5.73\ CO_2 + 4.68\ H_2O$$

$$(12*5.73)+(1*9.37)+(16*3.67) \qquad 6.23(16*2)$$

136.85 199.36

VS COD

COD/VS = 199.36/136.85 = 1.46 g COD/ g VS

TMP of food waste **=** 1.46 g COD/g VS * 350 ml CH_4 (STP) g COD

= 0.51 L CH_4 (STP)/ g VS

(iii) Methane yield based on elemental composition $C_{5.73}H_{9.37}O_{3.67}$

Using the Buswell's formula,

L CH_4 (STP)/ g VS $= \frac{\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}\right)22.4}{12n+a+16b}$

$= \frac{\left(\frac{5.73}{2}+\frac{9.37}{8}-\frac{3.67}{4}\right)22.4}{12(5.73)+9.37+16(3.67)}$

TMP of food waste **= 0.51 L CH4 (STP)/g VS**

Start-up Procedure

A preliminary experiment was conducted to investigate the successful way of starting-up a continuous reactor. The initial idea was for the experiments to proceed under high solids digestion. Purpose of the experiment was to see whether a reactor is to be initiated using a large amount of substrate (14 kg) with just minimal seed (400 ml) (Start-up 1 and 2) (refer Figure A1) or whether the substrate volume needs to be build up over time and start off with purely seed (14 L) (Start-up 3,4 and 5). The effect of alkali solution and nutrients addition during start-up was also examined.

The reactors which were half filled with substrates together with only 400 ml of seed saw reactors failed quickly. After just 24 hours, the reactors recorded a pH of 4.8 to 5.9. The Start-up 1 and 2 plot in Figure A2 demonstrates that the methane content for reactor treating food waste and meat, respectively was constantly below 10%.

Figure A1 Photo showing the reactor content at initiation loaded with 14 kg of meat and 400 ml of DSS

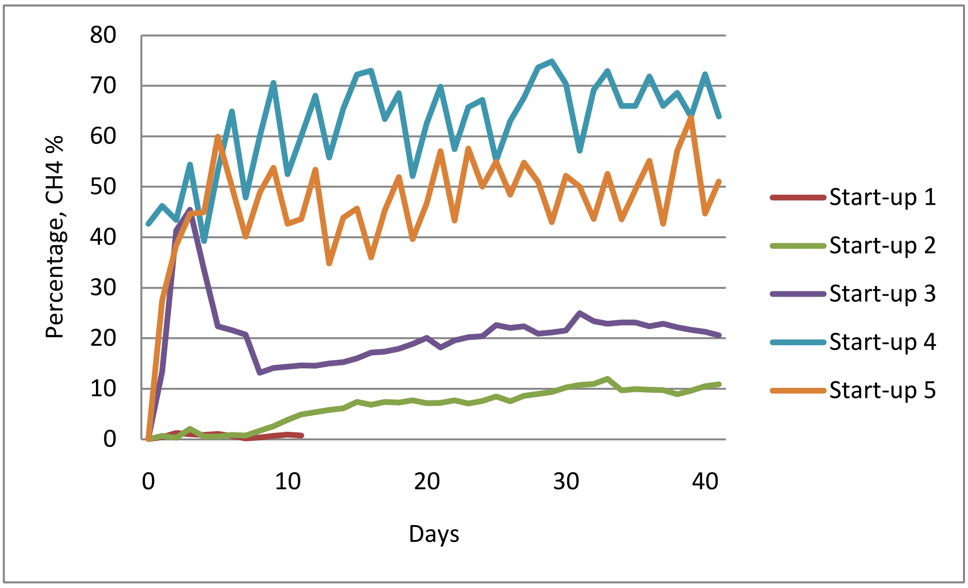


Figure A2 Performance of reactors applying Start-up procedure 1 to 5

A more promising response was observed when reactors were initiated purely on seed followed by regular substrate feeding. In Start-up 3, the methane percentage went to a maximum of 45% at Day 3 indicating that methane had begun to generate. Still, the methane production declined to an average of 20% thereafter. On Day 5, the pH of the reactor was 6.3 signifying an acidic condition. The initial burst of acid production and its accumulation may have led to the downturn in reactor performance.

When alkali solution and trace elements was added together in the reactor feed for Start-up 4 and 5, the pH values were 7.6 and 7.7, respectively at Day 4. At this point, the reactors produced more than 50% CH_4. The neutral pH values and high methane percentage seen with Start-up 4 and 5, signifies the effectiveness of buffer and trace elements addition at helping prevent acidic conditions to set in. This positive anaerobic decomposition for both Start-up 4 and 5 continued for 60 days of incubation.

It is therefore learnt that anaerobic digesters should be initiated using a large amount of seed and the substrate volume built up over time with regular feeding. Alkali solution and trace element is recommended during start-up to prevent process failure due to acid accumulation inhibiting methane production.

The determination of first order kinetic parameters for batch test; B0, k, t lag and SSR

Note: the following worked example is representative of Test label 1a in Table 6.2 of Chapter 6.

SSR	0.001	**T**
B0	0.44341	**U**
k	0.47454	**V**
t lag	0.63195	**W**

VS added = 20.24 g **X**

SAMPLE								**SEED**										
Gas Production					**Corrected to STP**			**Gas Production**				**Corrected to STP**			**P**	**Q**		
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**Corrected**	**CH4**	**R**	**S**
Day	% CH4	gas volume (ml)	CH4 daily (ml)	CH4 cum (ml)	Barometric Pressure (mbar)	(mmHg)	CH4 at STP (ml)	% CH4	gas volume (ml)	CH4 daily (ml)	CH4 cum (ml)	Barometric Pressure (mbar)	(mmHg)	CH4 at STP (ml)	**CH4 (ml)**	**Yield (L/ g VS)**	Fit Yield (L/ g VS)	
0	0.0	0.0	0.00	0.00	1010	757.78	0.00	0.0	0.0	0.00	0.00	1010	757.78	0.00	0.00	0.000		
1	40.8	4611.4	1881.46	1881.46	1010	757.78	1570.63	11.6	580.0	67.28	67.28	1010	757.78	56.16	1514.47	0.075	0.07106	0.000
2	55.2	5425.2	2994.71	4876.17	1009	757.03	4066.33	16.1	5.0	0.81	68.09	1009	757.03	56.78	4009.56	0.198	0.21174	0.000
3	68.7	4019.6	2761.45	7637.62	1002	751.78	6322.38	22.8	180.0	41.04	109.13	1002	751.78	90.33	6232.05	0.308	0.29927	0.000
4	68.9	1972.8	1359.26	8996.88	999	749.53	7423.95	25.7	215.0	55.26	164.38	999	749.53	135.64	7288.31	0.360	0.35373	0.000
5	67.3	1233.0	829.81	9826.69	1001	751.03	8125.88	28.4	130.0	36.92	201.30	1001	751.03	166.46	7959.42	0.393	0.38761	0.000
6	64.5	517.9	334.02	10160.71	997	748.03	8366.52	29.6	115.0	34.04	235.34	997	748.03	193.78	8172.74	0.404	0.40869	0.000
7	62.5	515.0	321.88	10482.58	997	748.03	8631.56	31.7	120.0	38.04	273.38	997	748.03	225.11	8406.45	0.415	0.42181	0.000
8	63.0	445.0	280.35	10762.93	1009	757.03	8975.42	34.2	100.0	34.20	307.58	1009	757.03	256.50	8718.92	0.431	0.42997	0.000
9	57.6	271.3	156.25	10919.18	1006	754.78	9077.05	29.2	55.0	16.06	323.64	1006	754.78	269.04	8808.01	0.435	0.43505	0.000
10	55.5	160.0	88.80	11007.98	992	744.28	9016.02	30.1	115.0	34.62	358.26	992	744.28	293.43	8722.59	0.431	0.4382	0.000
11	52.2	140.0	73.08	11081.06	990	742.78	9056.48	31.1	115.0	35.77	394.02	990	742.78	322.03	8734.45	0.432	0.44017	0.000
12	51.4	250.0	128.50	11209.56	987	740.53	9132.08	32.2	125.0	40.25	434.27	987	740.53	353.79	8778.29	0.434	0.44139	0.000
13	53.5	140.0	74.90	11284.46	998	748.78	9301.71	34.8	50.0	17.40	451.67	998	748.78	372.31	8929.41	0.441	0.44215	0.000
14	52.5	175.0	91.88	11376.33	1000	750.28	9397.36	35.6	10.0	3.56	455.23	1000	750.28	376.04	9021.32	0.446	0.44263	0.000
15	52.5	175.0	91.88	11468.21	1000	750.28	9473.25	35.6	10.0	3.56	458.79	1000	750.28	378.98	9094.27	0.449	0.44292	0.000
16	57.3	145.0	83.09	11551.29	988	741.28	9420.59	40.1	85.0	34.09	492.88	988	741.28	401.96	9018.63	0.446	0.4431	0.000
17	57.3	145.0	83.09	11634.38	988	741.28	9488.35	40.1	85.0	34.09	526.96	988	741.28	429.76	9058.59	0.448	0.44322	0.000
18	54.3	151.6	82.32	11716.70	977	733.02	9442.71	39.4	91.6	36.09	563.05	977	733.02	453.77	8988.93	0.444	0.44329	0.000
19	54.3	151.7	82.37	11799.07	977	733.02	9509.09	39.4	91.7	36.13	599.18	977	733.02	482.89	9026.20	0.446	0.44333	0.000
20	54.3	151.6	82.32	11881.39	977	733.02	9575.43	39.4	91.6	36.09	635.27	977	733.02	511.98	9063.46	0.448	0.44336	0.000

Columns A – Q are result from laboratory experiment

Columns:

A, B, C, F, I, J and M are data as measured from the tube headspace using the Gas Analyzer

D (methane volume sample, ml daily) = (B/100)*C

K (methane volume seed, ml daily) = (I/100)*K

E (methane volume sample, ml accumulated) = $D_n + D_{n+1}$

L (methane volume seed, ml accumulated) = $K_n + K_{n+1}$

G (barometric pressure in mmHg) = F*0.75028

N (barometric pressure in mmHg) = M*0.75028

H (methane volume, ml at STP) = E*(273/308)*((G-42)/760)

O (methane volume, ml at STP) =L*(273/308)*((N-42)/760)

P (corrected methane volume, ml) = methane volume of sample (H) – methane volume of seed (O)

Q (methane volume g VS added) = P/X

Columns R, S and cells T to W are estimated data

The steps taken to estimate the first order kinetics for a batch system using equation: $B = B_0*(1-e^{-k(t-tlag)})$ is as follows:

Columns:

R (methane volume g VS added) = U*(1-exp(-V*(A-W))

S (squared residual) = (Q – R)^2

T (sum of squared residual) = total S

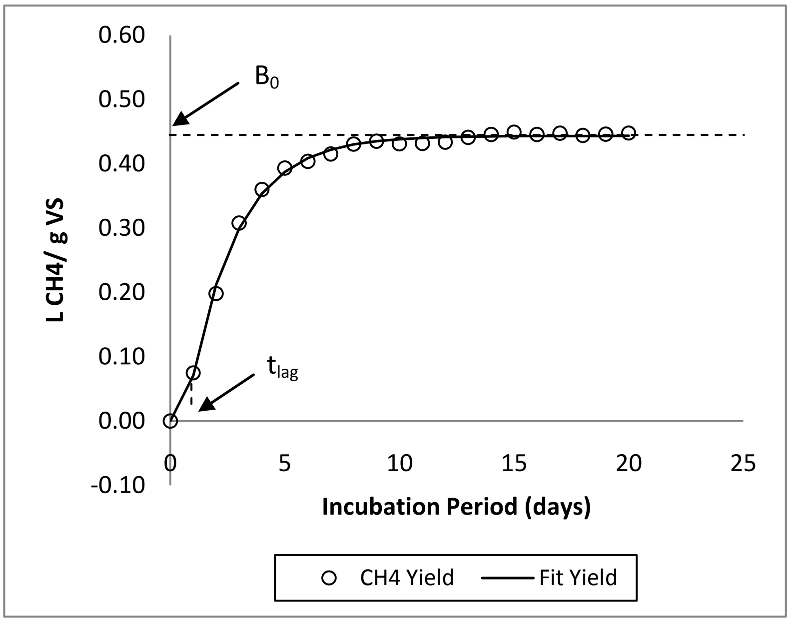
U (B0, L CH4/g VS) = may be estimated as the last data point from Q

V (k, d-1) = may be entered as any logical number

W (t lag, d) = may be estimated from the experimental data as graphed below

The parameters U, V and W (which are B0, k and t lag) are computed using the Solver function in the Excel worksheet by minimizing the T (sum of squared residual).

The experimental and estimated data is shown in graph below.



<u>The estimation of expected methane yield from the volatile solids reduction of food waste in tubes</u>

Note: the following worked example is representative of Test label 4b of Table 6.3 in Chapter 6. An explanation for each column will be given.

Table A2 The pH, experimental methane yield and volatile solids reduction of 18.8 OLR food waste digested in the tubes

Test	Measured Yield[a]	VS reduction	Total measured CH_4[b]	CH_4[c] not yet produced	Theoretical[d] CH_4 not measured	pH
	L CH_4/g VS	%	ml	ml	%	
4b	0.1410	90	2848	323	69	7.4
3a	0.1690	92	3423	302	62	7.3
3b	0.2440	100	4945	0	46	7.4
3c	0.2580	n/a	5215	n/a	43	7.5
4c	0.1550	97	3130	110	66	7.4
3d	0.3600	95	7283	408	20	7.4
3e	0.3930	93	7957	608	13	7.0
3f	0.4290	93	8692	674	5	7.4
2b	0.3800	91	7697	733	15	7.4
4a	0.3760	96	7612	325	16	7.5
1a	0.4480	70	9063	3487	0	7.5
2c	0.4680	90	9471	1064	**6**	7.4
2a	0.4990	93	10092	736	**0**	7.4

Note: a: methane per g VS as measured in the 20 day experiments; b: total volume of methane measured in the experiments; c: methane volume not yet produced (details for this calculation is given in Appendix 6b); The theoretical methane volume for food waste at 0.45 L CH_4/g VS is 9108 ml, while at 0.50 L CH_4/g VS is 10120 ml. d: the theoretical methane volume – b

Take 4b for example,

measured yield, L CH_4/g VS = 0.1410 ....of lab data

VS reduction, % = 90 ....of lab data

Total measured CH_4, ml = 2848 ....of lab data

CH_4 not yet produced, ml = 323

CH_4 not measured theoretically, % = 69

CH_4 (ml) not balanced is calculated considering the amount of methane that would be obtained if all of the VS added, which is 20.24 g, is turned into methane. For Test 4b, 90% of the VS added are reduced, meaning only 18.18 g of VS will be turned into methane and another 2.06 g VS is not. Nonetheless, in the laboratory, at the end of the 20 day incubation, 2848 ml CH_4 was measured. This means that **323 ml** of methane is not yet produced, calculated to the following example:

ml CH_4/g VS destroyed = 2848 ml CH_4/ (0.90*20.24) g VS destroyed

= 157

ml CH_4 not yet produced = ml CH_4/g VS destroyed*g VS not yet reduced

= 157 * (20.24-(0.90*20.24))

= 323

Theoretical CH_4 not measured is determined taking into account the actual methane that can be produced from a sample (food waste in this case). Theoretically 0.45 L CH_4 can be produced from a g VS food waste (refer Section 4.3.5, Chapter 4). However, the tube test established that for Test 4b, only 0.141 L CH_4 is achievable. This means instead of producing a maximum of 9108 ml CH_4 (20.24 g VS_{added}*0.45 L CH4/g VS_{added}), only 2848 ml CH4 was generated at the end of the test period. About **69%** (100-(2848/9108)*100) methane is theoretically is still obtainable from the food waste sample.

The under generation of methane both considering the VS destroyed and its theoretical potential for Test 4b signifies that (i) not all CH_4 was measured because of the 20 days incubation period instead of infinite time or (ii) process was inhibited for one reason or other.

Methane potential of food waste in bottles with modifications

The testing for batch bottles was executed in two phases. In the initial run no trace elements or buffer were supplemented in the digestion of 18.8 OLR food waste so that the conditions would be identical to the tube (results shown in Chapter 6). The later run described here was repeated at lower loading rates of 12.5 g VS/L. d and with several modifications. This includes the addition of buffer and/or trace element at varying concentrations and sample pre-treatment as specified in Table A3. For sample pre-treatment the food waste was dried overnight in an oven at 100°C followed by grinding using a mortar and sample to about 2 mm size after the sample has cooled down. The intention here is to identify any factors that could be limiting methane production in the batch bottles.

Table A3 Description of the modifications to the bottle conditions when digesting food waste at various OLR

Reactor label	OLR (g VS/L. d)	Supplementation	Concentration
T1 a, b	12.5	Trace element	22 ml (1%)
T2 a, b	12.5	Trace element	22 ml (5%)
B1 a, b	12.5	Phosphate buffer	1 ml (10%)
B2 a, b	12.5	Phosphate buffer	1.5 ml (10%)
B3 a, b	12.5	Phosphate buffer	3 ml (10%)
TB a, b	12.5	Trace element Phosphate buffer	22 ml (1 %) 1.5 ml (10%)
3 TB a, b	3.5	Trace element Phosphate buffer	22 ml (1 %) 1.5 ml (10%)
7 TB a, b	8.2	Trace element Phosphate buffer	22 ml (1 %) 1.5 ml (10%)
11 TB a, b	12.5	Trace element Phosphate buffer	22 ml (1 %) 1.5 ml (10%)
D TB a, b	12.5	Trace element Phosphate buffer Sample pre-treatment	22 ml (1 %) 1.5 ml (10%)
W TB a, b	12.5	Trace element	22 ml (1 %)

		Phosphate buffer	1.5 ml (10%)
D B3 a, b	12.5	Phosphate buffer Sample pre-treatment	3 ml (10%)
W B3 a, b	12.5	Phosphate buffer	3 ml (10%)
Control	12.5	Nil	Nil

Note: 1% trace element concentration means 2.5 ml trace element solution is added into 250 ml water. 5% trace element concentration means 12.5 ml trace element solution added into 250 ml water. 10% phosphate buffer concentration means 50 g potassium phosphate plus 50 g sodium hydroxide added into 1 L water

a) Effect of trace element addition

As depicted by Figure A3 the trace element when added to the bottles reduced the methane yield from 0.25 L CH_4/g VS to 0.17 L CH_4/g VS. A typical value of methane yield for food waste is between 0.35 to 0.55 L CH_4/g VS. This is seen at both low (T1a,b) and stronger (T2a,b) concentration of the trace elements.

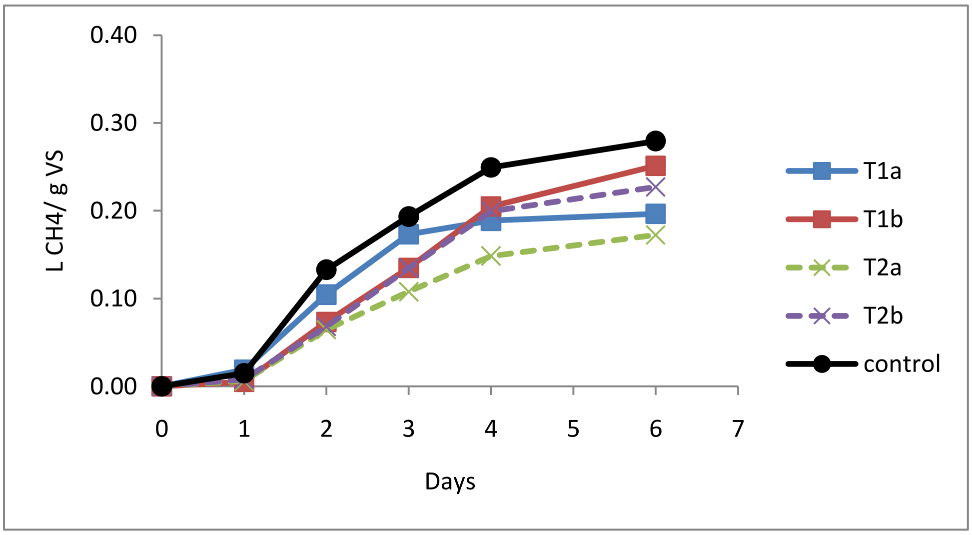


Figure A3 The effect of trace element addition to methane yield. T1a, b and T2 a, b are duplicates having similar substrate loading and conditions but a higher trace element concentration with the latter. Control is food waste digestion without trace element addition

b) Effect of buffer addition

When 1 ml of buffer is added to the bottles (B1a, b) the methane yield was down by 14 - 63% compared to if none were added (control) (refer Figure A4). Adding more buffer at 1.5 ml (B2a, b) increased the methane yield from 0.279 L CH_4/g VS (without supplementation) to a maximum of 0.312 L CH_4/g VS. However, a higher buffer volume of 3.0 ml will again lead to a reduced methane production. As shown by Figure A5 the methane yield dropped from 0.17 L CH_4/g VS to a lowest of 0.11 L CH_4/g VS with the augmented buffer volume of 3 ml.

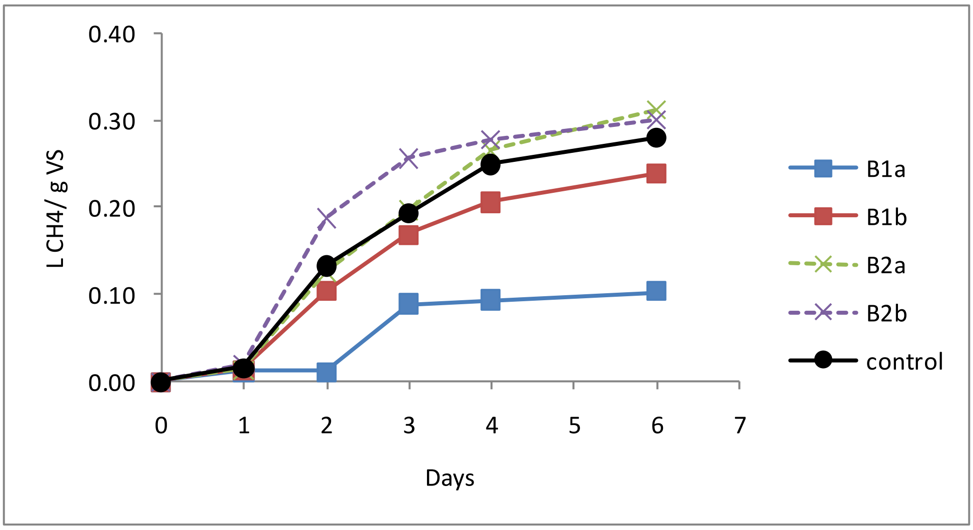


Figure A4 The effect of buffer addition to methane yield. B1a, b and B2 a, b are duplicates having similar substrate loading and conditions but at different buffer concentrations. B1a, b had 1 ml buffer added while B2a, b had 1.5 ml instead. Control is food waste digestion without buffering

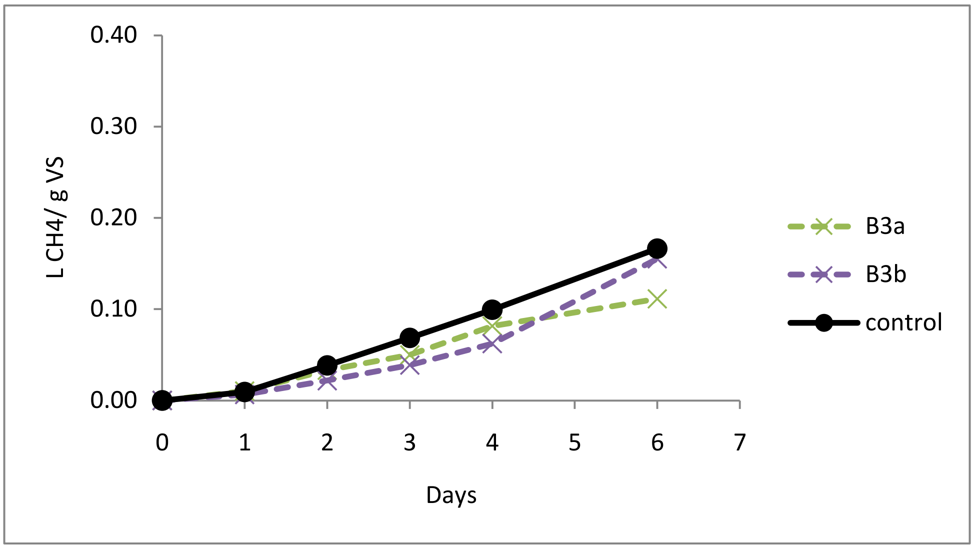


Figure A5 The effect of buffer addition at higher concentration (3 ml) to methane yield. Control is food waste digestion without buffering

c) Effect of adding trace element and buffer solution together

Concerned with the acidic trace element solution (pH 4.3) which could have led to the reduced methane yield, another test was repeated where 1.5 ml buffer was added. The result in Figure A6 reaffirms that adding trace element and buffer solution together in the bottles was not beneficial. This is further proven by Figure A7 where the varying OLR at 3.5, 8.2 and 12.9 g VS/L. d. also did not respond well to the inclusion of buffer and trace element. Instead digesting 12.9 g VS/L. d. OLR of food waste without any supplementation provided the highest methane yield of 0.22 L CH_4/g VS amongst them.

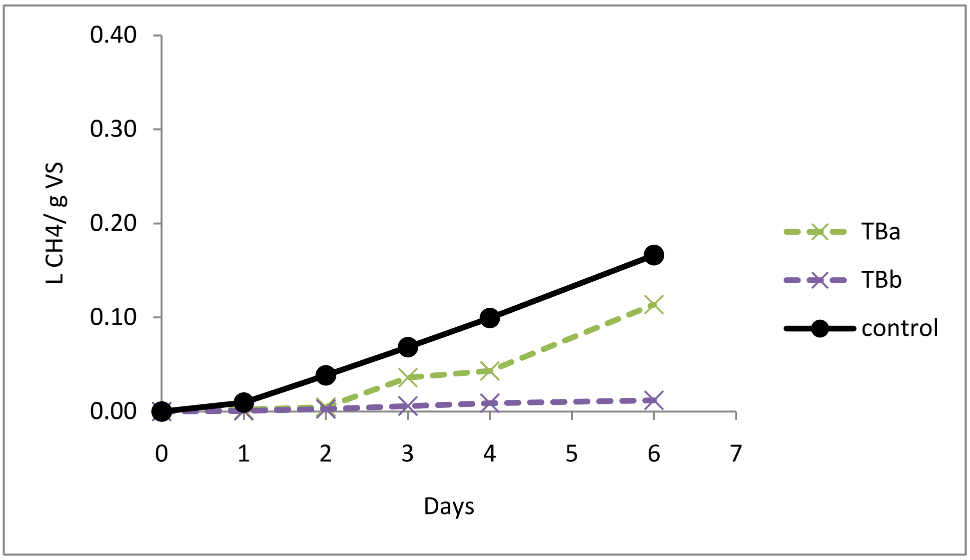


Figure A6 The effect of combining buffer and trace element to methane yield. The control is similar samples that are incubated without buffer and trace elements

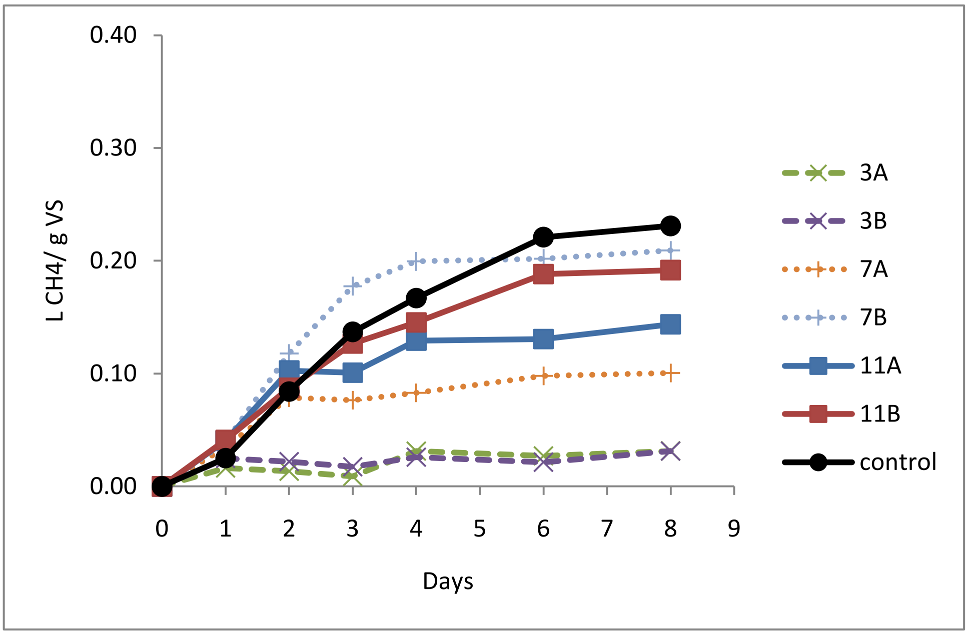


Figure A7 The effect of combining buffer and trace element to methane yield was further verified at various OLR. Duplicates were used at three OLRs of 3.53 g VS/L. d. (labelled as 3A, B), 8.23 g VS/L. d. (labelled as 7A, B) and 12.5 g VS/L. d. (labelled as 11A, B). Control is similar substrate incubated without supplementations at 12.5 g VS/L. d. OLR

Table A4 shows that ammonia averages at 881 (± 73) mg/l across the different food waste organic loading rates of 3.53, 8.23, and 12.5 g VS/L. d. The highest ammonia was 1040 mg/l, which is at concentrations that is considered safe for anaerobic process (refer Chapter 5). This is also the same for the SCOD where the concentrations between 775 to 1500 mg/l measured from the bottles is way below the levels that is indicative of an unstable reactor. Furthermore, the ammonia and SCOD of the food waste effluents in the bottles is still considerably lower than those recorded for food waste digestion in the tubes (refer Table 6.4 in Chapter 6). This is the same with the TVFA concentration that is lower than the method detection limit of 696 mg/l. Therefore, it is difficult to justify exactly what is restricting the methane production from a similar sample and OLR but incubated in the Schott bottles. The limiting factor could probably be more of a microbiological effect than biochemical reactions.

Table A4 Ammonia, soluble COD, pH and volatile fatty acids of 18.8 OLR food waste digested in the bottles taken at Day 20

Test	SNH_3-N (mg/l)	SCOD (mg/l)	pH	TVFA (mg/l)
3A	860	1275	8.1	< 696
3B	830	775	8.0	< 696
7A	860	1025	8.0	< 696
7B	795	900	8.0	< 696
11A	968	1525	8.0	< 696
11B	970	925	8.0	< 696
FW Control	1040	1200	8.0	< 696

d) Effect of sample pre-treatment

When investigating the significance of sample pre-treatment, the highest methane yield of 0.17 L CH_4/g VS was obvious from the control in Figures A8 and A9. The control consist of food waste sample at 12.5 g VS/L. d OLR which was not subjected to pre-treatment apart from blending for homogenization. In Figure A8 the second highest methane potential at

0.114 L CH_4/g VS was produced from one of the un-treated sample duplicate that were incubated along with trace elements and buffer. Samples that were dried and ground seemed to give out the least amount of methane yield with a maximum of only 0.077 L CH_4/g VS. Even so, pre-treating the samples before use looks as if it improved the reproducibility between duplicates. A yield of 0.051 L CH_4/g VS was obtained from the second replicate.

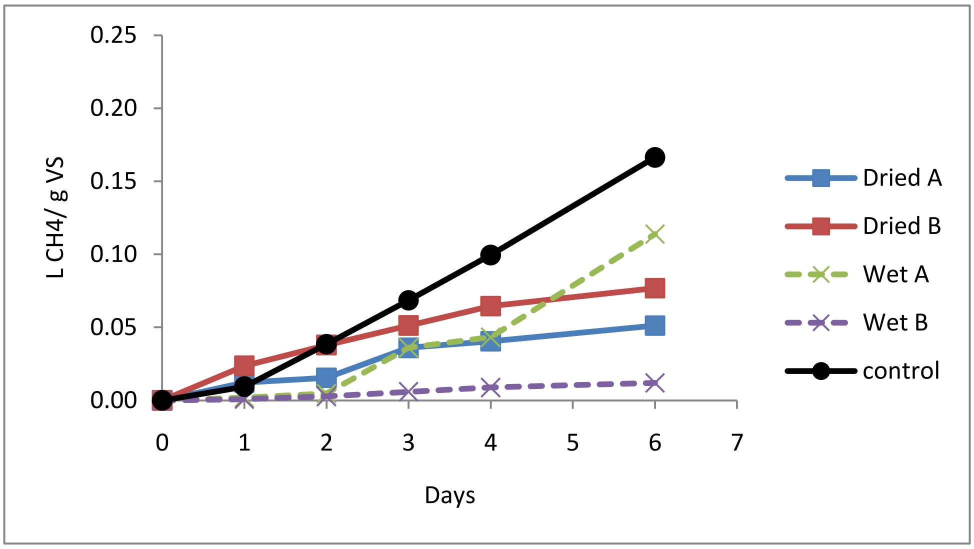


Figure A8 Food waste samples incubated at 12.5 g VS/L. d with (Dried A,B) and without sample pre-treatments (Wet A,B and control). All reactors had phosphate buffer and trace element added except for control.

The observations concerning the effect of sample pre-treatments are further strengthened by the repeated behaviour in Figure A9. Here, when solely buffer was added at 3 ml, the control again produced the highest methane yield. This was followed by the reactors that were using non pre-treated samples whereas the least yield was obtained from the reactors which dried and ground the samples first. An improved reproducibility between duplicate was again evident with the latter condition.

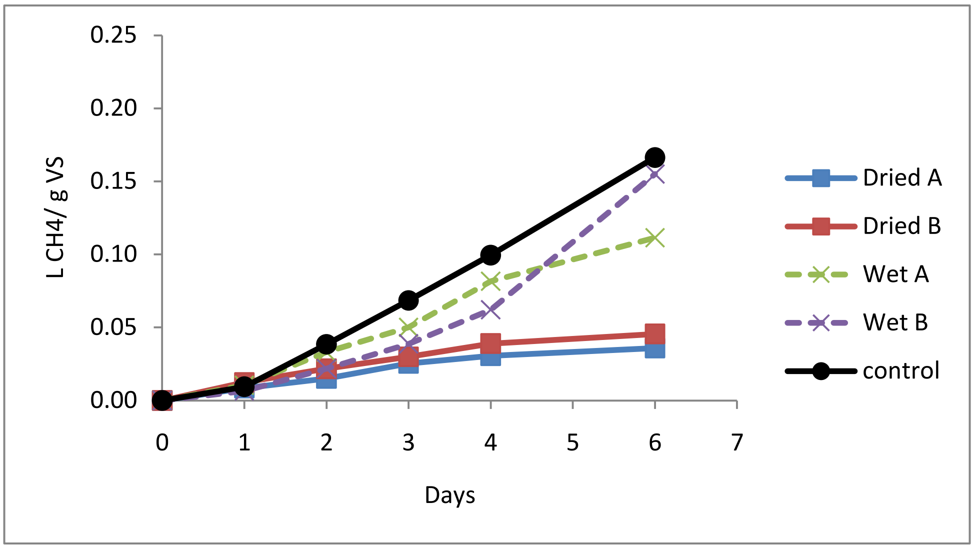


Figure A9 Food waste samples incubated at 12.5 g VS/L. d with (Dried A,B) and without sample pre-treatments (Wet A,B and control). All reactors had 3 ml phosphate buffer added except for control.

In conclusion, it is learnt that the methane potential of food waste is restricted in the bottles due to some other reasons. The addition of nutrients, buffer solution and sample pre-treatment did nothing to counter the limitation effect. Unfortunately, no concrete explanation could be given to as why methane potential of food waste could not be realised using the 250 ml Schott bottles. As shown in Section 6.3.1.1 of Chapter 6, the methane percentages for food waste in the bottles was extremely low, ranging between 0.7 - 28.9% after 20 days incubation. Souring did not occur as the pH was within neutral values (7.9 - 8.2). In comparison, for the same incubation period, food waste digested in the tubes was still producing about 62% methane. It seems methane generation in the bottles was not sustained.